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(54) Title: COMPOSITIONS AND METHODS FOR THE EARLY DIAGNOSIS OF OVARIAN CANCER

(57) Abstract: The disclosed nucleic acid primer sets, used in combination with quantitative amplification (PCR) of tissue cDNA, can indicate the presence of specific proteases in a tissue sample. The detected proteases are themselves specifically overexpressed in certain cancers, and their presence may serve for early detection of associated ovarian and other malignancies, and for the design of interactive therapies for cancer treatment.

COMPOSITIONS AND METHODS FOR THE EARLY DIAGNOSIS OF OVARIAN CANCER

BACKGROUND OF THE INVENTION

Cross-Reference to Related Applications

This application is a continuation-in-part and claims the benefit of priority under 35 USC §120 of USSN 09/039,211, filed March 14, 1998.

Field of the Invention

Generally, the present invention relates to the fields of molecular biology and medicine. More specifically, the present invention is in the field of ovarian and other cancer diagnosis.

Background of the Invention

To date, ovarian cancer remains the number one killer of women with gynecologic malignant hyperplasia. Approximately 75% of women diagnosed with such cancers are already at an advanced stage (III and IV) of the disease at their

initial diagnosis. During the past 20 years, neither diagnosis nor five year survival rates have greatly improved for these patients. This is substantially due to the high percentage of high-stage initial detections of the disease. Therefore, the challenge remains to develop new markers that improve early diagnosis and thereby reduce the percentage of high-stage initial diagnoses.

Extracellular proteases have already been implicated in the growth, spread and metastatic progression of many cancers, due to the ability of malignant cells not only to grow *in situ*, but to dissociate from the primary tumor and to invade new surfaces. The ability to disengage from one tissue and re-engage the surface of another tissue is what provides for the morbidity and mortality associated with this disease. Therefore, extracellular proteases may be good candidates for markers of neoplastic development.

In order for malignant cells to grow, spread or metastasize, they must have the capacity to invade local host tissue, dissociate or shed from the primary tumor, and for metastasis to occur, enter and survive in the bloodstream, implant by invasion into the surface of the target organ and establish an environment conducive for new colony growth (including the induction of angiogenic and growth factors). During this progression, natural tissue barriers have to be degraded, including basement membranes and connective tissue. These barriers include collagen, laminin, proteoglycans and extracellular matrix glycoproteins, including fibronectin. Degradation of these natural barriers, both those surrounding the primary tumor and at the sites of metastatic invasion, is believed

to be brought about by a matrix of extracellular proteases.

Proteases have been classified into four families: serine proteases, metallo-proteases, aspartic proteases and cysteine proteases. Many proteases have been shown to be involved in the human disease process and these enzymes are targets for the development of inhibitors as new therapeutic agents. Additionally, certain individual proteases have been shown to be induced and overexpressed in a diverse group of cancers, and as such, are potential candidates for markers of early diagnosis and possible therapeutic intervention. A group of examples are shown in Table 1.

TABLE 1

Known proteases expressed in various cancers

	Gastric	Brain	Breast	Ovarian
Serine Proteases:	uPA	uPA	NES-1	NES-1
	PAI-1	PAI-1	uPA	uPA
		tPA		PAI-2
Cysteine Proteases:	Cathepsin B	Cathepsin L	Cathepsin B	Cathepsin B
	Cathepsin L	Cathepsin L	Cathepsin L	
Metallo-proteases:	Matrilysin*	Matrilysin	Stromelysin-3	MMP-2
	Collagenase*	Stromelysin	MMP-8	
	Stromelysin-1*	Gelatinase B	MMP-9	
			Gelatinase A	

uPA, Urokinase-type plasminogen activator; tPA, Tissue-type plasminogen activator; PAI-I, Plasminogen activator 0 inhibitors; PAI-2, Plasminogen activator inhibitors; NES-1, Normal epithelial cell-specific-1; MMP, Matrix P metallo-protease. *Overexpressed in gastrointestinal ulcers.

Significantly, there is a good body of evidence supporting the downregulation or inhibition of individual

proteases and the reduction in invasive capacity or malignancy. In work by Clark *et al.*, inhibition of *in vitro* growth of human small cell lung cancer was demonstrated using a general serine protease inhibitor. More recently, Torres-Rosedo *et al.*, [*Proc.Natl.Acad.Sci.USA*, 90, 7181-7185 (1993)] demonstrated an inhibition of hepatoma tumor cell growth using specific antisense inhibitors for the serine protease hepsin gene. Metastatic potential of melanoma cells has also been shown to be reduced in a mouse model using a synthetic inhibitor (batimastat) of metallo-proteases. Powell *et al.* [*Cancer Research*, 53, 417-422 (1993)] presented evidence to confirm that the expression of extracellular proteases in relatively non-invasive tumor cells enhances their malignant progression using a tumorgenic, but non-metastatic, prostate cell line. Specifically, enhanced metastasis was demonstrated after introducing and expressing the PUMP-1 metallo-protease gene. There is also a body of data to support the notion that expression of cell surface proteases on relatively non-metastatic cell types increases the invasive potential of such cells.

Thus, the prior art is deficient in a good tumor marker useful as an indicator of early disease, particularly for ovarian cancers. The present invention fulfills this long-standing need and desire in the art.

SUMMARY OF THE INVENTION

This invention allows for the detection of cancer, especially ovarian cancer, by screening for PUMP-1 mRNA in

tissue, which is indicative of the PUMP-1 protease, which is shown herein to be specifically associated with the surface of 80 percent of ovarian and other tumors. Proteases are considered to be an integral part of tumor growth and metastasis, and therefore, markers indicative of their presence or absence are useful for the diagnosis of cancer. Furthermore, the present invention is useful for treatment (*i.e.*, by inhibiting PUMP-1 or expression of PUMP-1), for targeted therapy, for vaccination, etc.

In one embodiment of the present invention, there is provided a method of diagnosing cancer in an individual, comprising the steps of (a) obtaining a biological sample from an individual; and (b) detecting PUMP-1 in the sample. Usually, the presence of PUMP-1 in the sample is indicative of the presence of cancer in the individual, and the absence of PUMP-1 in the sample is indicative of the absence of cancer in the individual.

In one embodiment of the present invention, there is provided a method for detecting malignant hyperplasia in a biological sample, comprising the steps of (a) isolating mRNA from the sample; and (b) detecting PUMP-1 mRNA in the sample. Typically, the presence of the PUMP-1 mRNA in the sample is indicative of the presence of malignant hyperplasia, and the absence of the PUMP-1 mRNA in the sample is indicative of the absence of malignant hyperplasia.

In one embodiment of the present invention, there is provided a method for detecting malignant hyperplasia in a biological sample, comprising the steps of (a) isolating protein from the sample; and (b) detecting PUMP-1 protein in the sample. Generally, the presence of the PUMP-1 protein in the sample is

indicative of the presence of malignant hyperplasia, and the absence of the PUMP-1 protein in the sample is indicative of the absence of malignant hyperplasia.

In one embodiment of the present invention, there is provided a method of inhibiting expression of endogenous PUMP-1 in a cell, comprising the step of (a) introducing a vector into a cell, wherein the vector comprises a PUMP-1 gene in opposite orientation operably linked to elements necessary for expression. Upon expression of the vector, the cell produces PUMP-1 antisense mRNA, which hybridizes to endogenous PUMP-1 mRNA, thereby inhibiting expression of endogenous PUMP-1 in the cell.

In one embodiment of the present invention, there is provided a method of inhibiting PUMP-1 protein in a cell, comprising the step of (a) introducing an antibody into a cell which is specific for a PUMP-1 protein or a fragment thereof. Generally, binding of the antibody to the PUMP-1 protein inhibits the PUMP-1 protein.

In one embodiment of the present invention, there is provided a method of targeted therapy to an individual, comprising the step of (a) administering a compound to an individual, wherein the compound has a targeting moiety and a therapeutic moiety. Specifically, the targeting moiety is specific for PUMP-1.

In one embodiment of the present invention, there is provided a method of vaccinating an individual against PUMP-1, comprising the step of (a) inoculating an individual with a PUMP-1 protein or fragment thereof which lacks PUMP-1 protease activity. Typically, inoculation with the PUMP-1 protein or

fragment thereof elicits an immune response in the individual, thereby vaccinating the individual against PUMP-1.

In one embodiment of the present invention, there is provided a method of producing immune-activated cells directed toward PUMP-1, comprising the steps of exposing dendritic cells to a PUMP-1 protein or fragment thereof which lacks PUMP-1 protease activity. Usually, exposure to the PUMP-1 protein or fragment thereof activates the dendritic cells, thereby producing immune-activated cells directed toward PUMP-1.

In one embodiment of the present invention, there is provided an immunogenic composition, comprising an immunogenic fragment of a PUMP-1 protein and an appropriate adjuvant.

In one embodiment of the present invention, there is provided an oligonucleotide having a sequence complementary to SEQ ID No. 29, as well as a composition comprising the oligonucleotide and a physiologically acceptable carrier. Additionally, there is provided a method of treating a neoplastic state in an individual in need of such treatment, comprising the step of (a) administering to the individual an effective dose of the above-described oligonucleotide.

In one embodiment of the present invention, there is provided a method of screening for compounds that inhibit PUMP-1 activity, comprising the steps of (a) contacting a sample with a compound, wherein the sample comprises PUMP-1 protein; and (b) assaying for PUMP-1 protease activity. Typically, a decrease in PUMP-1 protease activity in the presence of the compound relative to PUMP-1 protease activity in the absence of

the compound is indicative of a compound that inhibits PUMP-1 activity.

In one embodiment of the present invention, there is provided a method for detecting ovarian malignant hyperplasia in a biological sample, comprising the steps of (a) isolating the proteases or protease mRNA present in the biological sample; and (b) detecting specific proteases or protease mRNA present in the biological sample. Representative proteases are hepsin, protease M, complement factor B, SCCE, other serine proteases indicated in lanes 2 and 4 of Figure 1, cathepsin L and PUMP-1.

Other and further aspects, features, and advantages of the present invention will be apparent from the following description of the presently preferred embodiments of the invention. These embodiments are given for the purpose of disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

The appended drawings have been included herein so that the above-recited features, advantages and objects of the invention will become clear and can be understood in detail. These drawings form a part of the specification. It is to be noted, however, that the appended drawings illustrate preferred embodiments of the invention and should not be considered to limit the scope of the invention.

Figure 1 shows agarose gel comparison of PCR products derived from normal and carcinoma cDNA.

Figure 2 shows Northern blot analysis of ovarian

tumors using hepsin, SCCE, PUMP-1, TADG-14 and β-tubulin probes.

Figure 3 shows amplification with serine protease redundant primers: histidine sense (S1) with aspartic acid antisense (AS1), using normal cDNA (Lane 1) and tumor cDNA (Lane 2); and histidine sense (S1) with serine antisense (AS2), using normal cDNA (Lane 3) and tumor cDNA (Lane 4).

Figure 4 shows amplification with cysteine protease redundant primers. Normal (Lane 1), low malignant potential (Lane 2), serious carcinoma (Lane 3), mucinous carcinoma (Lane 4), and clear cell carcinoma (Lane 5).

Figure 5 shows amplification with metallo-protease redundant primers. Normal (Lane 1), low malignant potential (Lane 2), serious carcinoma (Lane 3), mucinous carcinoma (Lane 4), and clear cell carcinoma (Lane 5).

Figure 6 shows amplification with specific primers directed towards the serine protease, hepsin. Expression in normal (Lanes 1-3), low malignant potential tumors (Lanes 4-8), and ovarian carcinomas (Lanes 9-12).

Figure 7 shows hepsin expression levels in normal, low malignant potential tumors, and ovarian carcinomas. S=serious, M=mucinous, LMP=low malignant potential.

Figure 8 shows serine protease stratum corneum chymotrypsin enzyme (SCCE) expression in normal, low malignant potential tumors, and ovarian carcinomas.

Figure 9 shows metallo-protease PUMP-1 (MMP-7) gene expression in normal (lanes 1-2) and ovarian carcinomas tissue (Lanes 3-10).

Figure 10 shows Northern blot analysis of hepsin expression in normal ovary and ovarian carcinomas. **(Figure 10A)**, *lane 1*, normal ovary (case 10); *lane 2*, serous carcinoma (case 35); *lane 3*, mucinous carcinoma (case 48); *lane 4*, endometrioid carcinoma (case 51); and *lane 5*, clear cell carcinoma (case 54). In cases 35, 51 and 54, more than a 10-fold increase in the hepsin 1.8 kb transcript abundance was observed. Northern blot analysis of hepsin in normal human fetal **(Figure 10B)** and adult tissues **(Figure 10C)**. Significant overexpression of the hepsin transcript is noted in both fetal liver and fetal kidney. Notably, hepsin overexpression is not observed in normal adult tissue. Slight expression above the background level is observed in the adult prostate.

Figure 11 shows hepsin expression in normal (N), mucinous (M) and serous (S) low malignant potential (LMP) tumors and carcinomas (CA). **Figure 11A** shows quantitative PCR of hepsin and internal control β-tubulin. **Figure 11B** shows a bar graph of expression of hepsin in 10 normal ovaries and 44 ovarian carcinoma samples.

Figure 12 shows northern blot analysis of mRNA expression of the SCCE gene in fetal tissue **(Figure 12A)** and in ovarian tissue **(Figure 12B)**.

Figure 13A shows a comparison of quantitative PCR of cDNA from normal ovary and ovarian carcinomas. **Figure 13B** shows a bar graph comparing the ratio of SCCE to β-tubulin in 10 normal and 44 ovarian carcinoma tissues.

Figure 14 shows a comparison by quantitative PCR of normal and ovarian carcinoma expression of mRNA for protease

M.

Figure 15 shows the TADG-12 catalytic domain including an insert near the His 5'-end.

Figure 16 shows northern blot analysis comparing TADG-14 expression in normal and ovarian carcinoma tissues (**Figure 16A**), and preliminary quantitative PCR amplification of normal and carcinoma cDNAs using specific primers for TADG-14 (**Figure 16B**).

Figure 17A shows Northern blot analysis of PUMP-1 mRNA from normal ovary and ovarian carcinomas. *Lane 1*, normal ovary; *lane 2*, serous carcinoma; *lane 3*, mucinous carcinoma; *lane 4*, endometrioid carcinoma; *lane 5*, clear cell carcinoma. PUMP-1 transcripts are detected only in carcinoma cases (lanes 2-5). **Figure 17B** shows that among normal human fetal tissues, fetal lung and fetal kidney show increased transcript. **Figure 17C** shows that PUMP-1 overexpression is not observed in normal human adult tissues. Slight expression above the background level is observed in the prostate.

Figure 18A shows quantitative PCR analysis of PUMP-1 expression. Cases 3, 4 an d9 are normal ovaries. Cases 19. 21, 14, 15 and 16 are LMP tumors. Cases 43, 23, 36 and 37 are ovarian carcinomas. Expression levels of PUMP-1 relative to β-tubulin are significantly elevated in 8 or 9 tumor cases compared to that or normal ovaries. **Figure 18B** shows the ratio of mRNA expression of PUMP-1 compared to the internal control β-tubulin in 10 normal and 44 ovarian carcinomas.

Figure 19 shows the ratio of PUMP-1 expression to expression of β-tubulin in normal ovary, LMP tumors and ovarian

carcinomas. PUMP-1 mRNA expression levels were significantly elevated in LMP tumor ($p<0.05$) and carcinoma ($p<0.0001$) compared to that in normal ovary. All 10 samples of individual normal ovary showed low levels of PUMP-1 expression.

Figure 20 shows immunohistochemical staining of PUMP-1 in normal ovary and ovarian tumor tissues. **Figure 20A** shows normal ovarian epithelium shows no PUMP-1 immunoreactivity (x20). **Figure 20B** shows intense staining of secretory vessels in mucinous tumors (x20). **Figure 20C** shows cytoplasmic staining of PUMP-1 in serous tumors (x20). **Figure 20D** shows clear cell tumors (x100). **Figure 20E** shows secretion of PUMP-1 to the extracellular environment of endometrioid tumors (x100).

Figure 21 shows a comparison of PCR amplified products for the hepsin, SCCE, protease M, PUMP-1 and Cathepsin L genes.

DETAILED DESCRIPTION OF THE INVENTION

This invention identifies a PUMP-1 protease on ovarian and other tumor cells which is characteristic of this type of cancer, and in various combinations with other proteases, is characteristic of individual tumor types. Such information can provide the basis for diagnostic tests (assays or immunohistochemistry), prognostic evaluation (depending on the display pattern) and therapeutic intervention utilizing either antibodies directed at the protease, antisense vehicles for downregulation or protease inhibitors both from established

inhibition data and/or for the design of new drugs. Long-term treatment of tumor growth, invasion and metastasis has not succeeded with existing chemotherapeutic agents - most tumors become resistant to drugs after multiple cycles of chemotherapy. The full-length sequence of PUMP-1 (SEQ ID No. 29) is as follows:

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   1 AAGAACAATT GTCTCTGGAC GGCAGCTATG CGACTCACCG TGCTGTGTGC
  51 TGTGTGCCTG CTGCCTGGCA GCCTGGCCCT GCCGCTGCCT CAGGAGGCGG
 101 GAGGCATGAG TGAGCTACAG TGGGAACAGG CTCAGGACTA TCTCAAGAGA
 151 TTTTATCTCT ATGACTCAGA AACAAAAAAT GCCAACAGTT TAGAAGCCAA
 201 ACTCAAGGAG ATGCAAAAAT TCTTTGGCCT ACCTATAACT GGAATGTTAA
 251 ACTCCCGCGT CATAGAAATA ATGCAGAAGC CCAGATGTGG AGTGCCAGAT
 301 GTTGCAGAAT ACTCACTATT TCCAAATAGC CCAAAATGGA CTTCCAAAGT
 351 GGTCACCTAC AGGATCGTAT CATATACTCG AGACTTACCG CATATTACAG
 401 TGGATCGATT AGTGTCAAAG GCTTTAAACA TGTGGGGCAA AGAGATCCCC
 451 CTGCATTTCA GGAAAGTTGT ATGGGGAACT GCTGACATCA TGATTGGCTT
 501 TGCGCGAGGA GCTCATGGGG ACTCCTACCC ATTTGATGGG CCAGGAAACA
 551 CGCTGGCTCA TGCCTTTGCG CCTGGGACAG GTCTCGGAGG AGATGCTCAC
 601 TTCGATGAGG ATGAACGCTG GACGGATGGT AGCAGTCTAG GGATTAACTT
 651 CCTGTATGCT GCAACTCATG AACTTGGCCA TTCTTTGGGT ATGGGACATT
 701 CCTCTGATCC TAATGCAGTG ATGTATCCAA CCTATGGAAA TGGAGATCCC
 751 CAAAATTTTA AACTTTCCCA GGATGATATT AAAGGCATTC AGAAACTATA
 801 TGGAAAGAGA AGTAATTCAA GAAAGAAATA GAAACTTCAG GCAGAACATC
 851 CATTCATTCA TTCATTGGAT TGTATATCAT TGTTGCACAA TCAGAATTGA
 901 TAAGCACTGT TCCTCCACTC CATTTAGCAA TTATGTCACC CTTTTTTATT
 951 GCAGTTGGTT TTTGAATGTC TTTCACTCCT TTTATTGGTT AAACTCCTTT
1001 ATGGTGTGAC TGTGTCTTAT TCCATCTATG AGCTTTGTCA GTGCGCGTAG
1051 ATGTCAATAA ATGTTACATA CACAAATA
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The present invention is directed toward a method of diagnosing cancer in an individual, comprising the steps of (a) obtaining a biological sample from an individual; and (b) detecting PUMP-1 in the sample. Generally, the presence of PUMP-1 in the sample is indicative of the presence of cancer in

the individual, and the absence of PUMP-1 in the sample is indicative of the absence of cancer in the individual.

The present invention is directed toward a method for detecting malignant hyperplasia in a biological sample, comprising the steps of (a) isolating mRNA from the sample; and (b) detecting PUMP-1 mRNA in the sample. Typically, the presence of the PUMP-1 mRNA in the sample is indicative of the presence of malignant hyperplasia, and the absence of the PUMP-1 mRNA in the sample is indicative of the absence of malignant hyperplasia. This method may further comprise the step(s) of comparing the PUMP-1 mRNA to reference information, wherein the comparison provides a diagnosis of the malignant hyperplasia; and/or comparing the PUMP-1 mRNA to reference information, wherein the comparison determines a treatment of the malignant hyperplasia. Preferably, detection of the PUMP-1 mRNA is by PCR amplification, and even more preferably, using primers SEQ ID No. 8 and SEQ ID No. 9.

The present invention is directed toward a method for detecting malignant hyperplasia in a biological sample, comprising the steps of (a) isolating protein from the sample; and (b) detecting PUMP-1 protein in the sample. Typically, the presence of the PUMP-1 protein in the sample is indicative of the presence of malignant hyperplasia, and the absence of the PUMP-1 protein in the sample is indicative of the absence of malignant hyperplasia. This method may further comprise the step(s) of comparing the PUMP-1 protein to reference information, wherein the comparison provides a diagnosis of the malignant hyperplasia; and/or comparing the PUMP-1 protein to reference

information, wherein the comparison determines a treatment of the malignant hyperplasia. Typically, detection is by immunoaffinity to an antibody, wherein the antibody is specific for PUMP-1.

The present invention is directed toward a method of inhibiting expression of endogenous PUMP-1 in a cell, comprising the step of (a) introducing a vector into a cell, wherein the vector comprises a PUMP-1 gene in opposite orientation operably linked to elements necessary for expression. Upon expression of the vector in the cell, PUMP-1 antisense mRNA is produced, which hybridizes to endogenous PUMP-1 mRNA, thereby inhibiting expression of endogenous PUMP-1 in the cell.

The present invention is directed toward a method of inhibiting PUMP-1 protein in a cell, comprising the step of (a) introducing an antibody into a cell, which is specific for a PUMP-1 protein or a fragment thereof, whereupon binding of the antibody to the PUMP-1 protein inhibits the PUMP-1 protein.

The present invention is directed toward a method of targeted therapy to an individual, comprising the step of (a) administering a compound to an individual, wherein the compound has a targeting moiety and a therapeutic moiety. In the present invention, the targeting moiety is specific for PUMP-1. Preferably, the targeting moiety is an antibody specific for PUMP-1 or a ligand or ligand binding domain that binds PUMP-1. Likewise, the therapeutic moiety is preferably a radioisotope, a toxin, a chemotherapeutic agent, an immune stimulant or a cytotoxic agent. Generally, the individual suffers from a disease such as ovarian cancer, lung cancer, prostate cancer, colon

cancer or another cancer in which PUMP-1 is overexpressed.

The present invention is directed toward a method of vaccinating an individual against PUMP-1, comprising the step of (a) inoculating an individual with a PUMP-1 protein or fragment thereof, which lacks PUMP-1 protease activity. Generally, inoculation with the PUMP-1 protein or fragment thereof elicits an immune response in the individual, thereby vaccinating the individual against PUMP-1. Typically, the individual has cancer, is suspected of having cancer or is at risk of getting cancer. Preferably, the PUMP-1 fragment is a 9-residue fragment up to a 20-residue fragment, and more preferably, the 9-residue fragment is SEQ ID Nos. 30, 31, 32, 33, 50, 70, 110, 111, 150, 151 or 152.

The present invention is directed toward a method of producing immune-activated cells directed toward PUMP-1, comprising the steps of exposing dendritic cells to a PUMP-1 protein or fragment thereof, which lacks PUMP-1 protease activity. Typically, exposure to the PUMP-1 protein or fragment thereof activates the dendritic cells, thereby producing immune-activated cells directed toward PUMP-1. Generally, the immune-activated cells are B-cells, T-cells and/or dendrites. Preferably, the PUMP-1 fragment is a 9-residue fragment up to a 20-residue fragment, and more preferably, the 9-residue fragment is SEQ ID Nos. 30, 31, 32, 33, 50, 70, 110, 111, 150, 151 or 152. Oftentimes, the dendritic cells are isolated from an individual prior to exposure and then reintroduced into the individual subsequent to the exposure. Typically, the individual has cancer, is suspected of having cancer or is at risk of getting cancer.

The present invention is directed toward an immunogenic composition, comprising an immunogenic fragment of a PUMP-1 protein and an appropriate adjuvant. Preferably, the fragment is a 9-residue fragment up to a 20-residue fragment, and more preferably, the 9-residue fragment is SEQ ID Nos. 30, 31, 32, 33, 50, 70, 110, 111, 150, 151 or 152.

The present invention is directed toward an oligonucleotide having a sequence complementary to SEQ ID No. 29. The present invention further provides a composition comprising the above-described oligonucleotide and a physiologically acceptable carrier. The present invention is directed toward a method of treating a neoplastic state in an individual in need of such treatment, comprising the step of (a) administering to the individual an effective dose of the above-described oligonucleotide. Typically, the neoplastic state is ovarian cancer, breast cancer, lung cancer, colon cancer, prostate cancer or another cancer in which PUMP-1 is overexpressed.

The present invention is directed toward a method of screening for compounds that inhibit PUMP-1 activity, comprising the steps of (a) contacting a sample with a compound, wherein the sample comprises PUMP-1 protein; and (b) assaying for PUMP-1 protease activity. Usually, a decrease in the PUMP-1 protease activity in the presence of the compound relative to PUMP-1 protease activity in the absence of the compound is indicative of a compound that inhibits PUMP-1 activity.

The present invention is directed toward a method for detecting ovarian malignant hyperplasia in a biological sample,

comprising the steps of (a) isolating the proteases or protease mRNA present in the biological sample; and (b) detecting specific proteases or protease mRNA present in the biological sample. Representative proteases are hepsin, protease M, complement factor B, SCCE, other serine proteases indicated in lanes 2 and 4 of Figure 1, cathepsin L and PUMP-1. This method may further comprise the step of comparing the specific proteases or protease mRNA detected to reference information, wherein the comparison provides a diagnoses of the malignant hyperplasia; and/or comparing the specific proteases or protease mRNA detected to reference information, wherein the comparison determines a treatment of the malignant hyperplasia. Generally, the protease mRNA is detected by amplification of total mRNA, or the protease is detected with an antibody.

According to the above descriptions of the present invention, representative biological samples are blood, urine, saliva, tears, interstitial fluid, ascites fluid, tumor tissue biopsy and circulating tumor cells. Typically, detection of the PUMP-1 is by means such as Northern blot, Western blot, PCR, dot blot, ELIZA sandwich assay, radioimmunoassay, DNA array chips and flow cytometry. Generally, the cancer is ovarian, breast, lung, colon, prostate or others in which PUMP-1 is overexpressed.

An additional object of the present invention is a number of nucleic acid sequences that are useful in its practice. These nucleic acid sequences are listed in Table 2. It is anticipated that these nucleic acid sequences be used in mixtures to accomplish the utility of this invention. Features of such mixtures include: SEQ ID Nos. 1 & 2; SEQ ID Nos. 1 & 3; SEQ ID

Nos. 4 & 5; SEQ ID Nos. 6 & 7; and SEQ ID Nos. 8 & 9. The skilled artisan may be able to develop other nucleic acid sequences and mixtures thereof to accomplish the benefit of this invention, but it is advantageous to have the sequences listed in Table 2 available without undue experimentation.

It will be apparent to one skilled in the art that various substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, *e.g.*, Maniatis, Fritsch & Sambrook, "Molecular Cloning: A Laboratory Manual (1982); "DNA Cloning: A Practical Approach," Volumes I and II (D.N. Glover ed. 1985); "Oligonucleotide Synthesis" (M.J. Gait ed. 1984); "Nucleic Acid Hybridization" (B.D. Hames & S.J. Higgins eds. 1985); "Transcription and Translation" (B.D. Hames & S.J. Higgins eds. 1984); "Animal Cell Culture" (R.I. Freshney, ed. 1986); "Immobilized Cells And Enzymes" (IRL Press, 1986); B. Perbal, "A Practical Guide To Molecular Cloning" (1984).

Therefore, if appearing herein, the following terms shall have the definitions set out below.

As used herein, the term "cDNA" shall refer to the DNA copy of the mRNA transcript of a gene.

As used herein, the term "derived amino acid sequence" shall mean the amino acid sequence determined by reading the triplet sequence of nucleotide bases in the cDNA.

As used herein the term “screening a library” shall refer to the process of using a labeled probe to check whether, under the appropriate conditions, there is a sequence complementary to the probe present in a particular DNA library. In addition, “screening a library” could be performed by PCR.

As used herein, the term “PCR” refers to the polymerase chain reaction that is the subject of U.S. Patent Nos. 4,683,195 and 4,683,202 to Mullis, as well as other improvements now known in the art.

The amino acid described herein are preferred to be in the "L" isomeric form. However, residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the desired functional property of immunoglobulin-binding is retained by the polypeptide. NH_2 refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxy terminus of a polypeptide. In keeping with standard polypeptide nomenclature, *J Biol. Chem.*, 243:3552-59 (1969), abbreviations for amino acid residues may be used.

It should be noted that all amino-acid residue sequences are represented herein by formulae whose left and right orientation is in the conventional direction of amino-terminus to carboxy-terminus. Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino-acid residues. The above Table is presented to correlate the three-letter and one-letter notations which may appear alternately herein.

A "replicon" is any genetic element (*e.g.*, plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication *in vivo*; *i.e.*, capable of replication under its own control.

A "vector" is a replicon, such as plasmid, phage or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment. A "vector" may further be defined as a replicable nucleic acid construct, *e.g.*, a plasmid or viral nucleic acid.

A "DNA molecule" refers to the polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in its either single-stranded form or as a double-stranded helix. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear DNA molecules (*e.g.*, restriction fragments), viruses, plasmids, and chromosomes. The structure is discussed herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (*i.e.*, the strand having a sequence homologous to the mRNA).

An expression vector is a replicable construct in which a nucleic acid sequence encoding a polypeptide is operably linked to suitable control sequences capable of effecting expression of the polypeptide in a cell. The need for such control sequences will vary depending upon the cell selected and the transformation method chosen. Generally, control sequences include a transcriptional promoter and/or enhancer, suitable

mRNA ribosomal binding sites and sequences which control the termination of transcription and translation. Methods which are well known to those skilled in the art can be used to construct expression vectors containing appropriate transcriptional and translational control signals. See, for example, techniques described in Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual* (2nd Ed.), Cold Spring Harbor Press, N.Y. A gene and its transcription control sequences are defined as being "operably linked" if the transcription control sequences effectively control transcription of the gene. Vectors of the invention include, but are not limited to, plasmid vectors and viral vectors. Preferred viral vectors of the invention are those derived from retroviruses, adenovirus, adeno-associated virus, SV40 virus, or herpes viruses. In general, expression vectors contain promoter sequences which facilitate the efficient transcription of the inserted DNA fragment and are used in connection with a specific host. The expression vector typically contains an origin of replication, promoter(s), terminator(s), as well as specific genes which are capable of providing phenotypic selection in transformed cells. The transformed hosts can be fermented and cultured according to means known in the art to achieve optimal cell growth.

An "origin of replication" refers to those DNA sequences that participate in DNA synthesis.

A DNA "coding sequence" is a double-stranded DNA sequence which is transcribed and translated into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are typically

determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (*e.g.*, mammalian) DNA, and even synthetic DNA sequences. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, polyadenylation signals, terminators, and the like, that provide for the expression of a coding sequence in a host cell.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site, as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters often, but not always, contain "TATA" boxes and "CAT" boxes. Prokaryotic promoters typically contain Shine-Dalgarno ribosome-binding sequences in addition to the -10 and -35 consensus sequences.

An "expression control sequence" is a DNA sequence

that controls and regulates the transcription and translation of another DNA sequence. A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then translated into the protein encoded by the coding sequence.

A "signal sequence" can be included near the coding sequence. This sequence encodes a signal peptide, N-terminal to the polypeptide, that communicates to the host cell to direct the polypeptide to the cell surface or secrete the polypeptide into the media, and this signal peptide is clipped off by the host cell before the protein leaves the cell. Signal sequences can be found associated with a variety of proteins native to prokaryotes and eukaryotes.

As used herein, the terms "restriction endonucleases" and "restriction enzymes" refer to enzymes, each of which cut double-stranded DNA at or near a specific nucleotide sequence.

A cell has been "transformed" by exogenous or heterologous DNA when such DNA has been introduced inside the cell. The transforming DNA may or may not be integrated (covalently linked) into the genome of the cell. In prokaryotes, yeast, and mammalian cells for example, the transforming DNA may be maintained on an episomal element such as a plasmid. With respect to eukaryotic cells, a stably transformed cell is one in which the transforming DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones

comprised of a population of daughter cells containing the transforming DNA. A "clone" is a population of cells derived from a single cell or ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth *in vitro* for many generations.

Two DNA sequences are "substantially homologous" when at least about 75% (preferably at least about 80%, and most preferably at least about 90% or 95%) of the nucleotides match over the defined length of the DNA sequences. Sequences that are substantially homologous can be identified by comparing the sequences using standard software available in sequence data banks, or in a Southern hybridization experiment under, for example, stringent conditions as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, *e.g.*, Maniatis et al., *supra*; DNA Cloning, Vols. I & II, *supra*; Nucleic Acid Hybridization, *supra*.

A "heterologous" region of the DNA construct is an identifiable segment of DNA within a larger DNA molecule that is not found in association with the larger molecule in nature. Thus, when the heterologous region encodes a mammalian gene, the gene will usually be flanked by DNA that does not flank the mammalian genomic DNA in the genome of the source organism. Another example is a construct where the coding sequence itself is not found in nature (*e.g.*, a cDNA where the genomic coding sequence contains introns, or synthetic sequences having codons different than the native gene). Allelic variations or naturally-occurring mutational events do not give rise to a heterologous region of DNA as defined herein.

The labels most commonly employed for these studies are radioactive elements, enzymes, chemicals which fluoresce when exposed to ultraviolet light, and others. A number of fluorescent materials are known and can be utilized as labels. These include, for example, fluorescein, rhodamine, auramine, Texas Red, AMCA blue and Lucifer Yellow. A particular detecting material is anti-rabbit antibody prepared in goats and conjugated with fluorescein through an isothiocyanate. Proteins can also be labeled with a radioactive element or with an enzyme. The radioactive label can be detected by any of the currently available counting procedures. The preferred isotope may be selected from ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{36}Cl, ^{51}Cr, ^{57}Co, ^{58}Co, ^{59}Fe, ^{90}Y, ^{125}I, ^{131}I, and ^{186}Re. Enzyme labels are likewise useful, and can be detected by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques. The enzyme is conjugated to the selected particle by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde and the like. Many enzymes which can be used in these procedures are known and can be utilized. The preferred are peroxidase, β-glucuronidase, β-D-glucosidase, β-D-galactosidase, urease, glucose oxidase plus peroxidase and alkaline phosphatase. U.S. Patent Nos. 3,654,090, 3,850,752, and 4,016,043 are referred to by way of example for their disclosure of alternate labeling material and methods.

A particular assay system developed and utilized in the art is known as a receptor assay. In a receptor assay, the material to be assayed is appropriately labeled and then certain cellular test colonies are inoculated with a quantitiy of both the

label after which binding studies are conducted to determine the extent to which the labeled material binds to the cell receptors. In this way, differences in affinity between materials can be ascertained.

An assay useful in the art is known as a "cis/trans" assay. Briefly, this assay employs two genetic constructs, one of which is typically a plasmid that continually expresses a particular receptor of interest when transfected into an appropriate cell line, and the second of which is a plasmid that expresses a reporter such as luciferase, under the control of a receptor/ligand complex. Thus, for example, if it is desired to evaluate a compound as a ligand for a particular receptor, one of the plasmids would be a construct that results in expression of the receptor in the chosen cell line, while the second plasmid would possess a promoter linked to the luciferase gene in which the response element to the particular receptor is inserted. If the compound under test is an agonist for the receptor, the ligand will complex with the receptor, and the resulting complex will bind the response element and initiate transcription of the luciferase gene. The resulting chemiluminescence is then measured photometrically, and dose response curves are obtained and compared to those of known ligands. The foregoing protocol is described in detail in U.S. Patent No. 4,981,784.

As used herein, the term "host" is meant to include not only prokaryotes but also eukaryotes such as yeast, plant and animal cells. A recombinant DNA molecule or gene which encodes a human PUMP-1 protein of the present invention can be used to transform a host using any of the techniques commonly

known to those of ordinary skill in the art. Especially preferred is the use of a vector containing coding sequences for the gene which encodes a human PUMP-1 protein of the present invention for purposes of prokaryote transformation. Prokaryotic hosts may include *E. coli*, *S. tymphimurium*, *Serratia marcescens* and *Bacillus subtilis*. Eukaryotic hosts include yeasts such as *Pichia pastoris*, mammalian cells and insect cells.

As used herein, "substantially pure DNA" means DNA that is not part of a milieu in which the DNA naturally occurs, by virtue of separation (partial or total purification) of some or all of the molecules of that milieu, or by virtue of alteration of sequences that flank the claimed DNA. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote; or which exists as a separate molecule (*e.g.*, a cDNA or a genomic or cDNA fragment produced by polymerase chain reaction (PCR) or restriction endonuclease digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence, *e.g.*, a fusion protein. Also included is a recombinant DNA which includes a portion of the nucleotides listed in SEQ ID No. 29 and which encodes an alternative splice variant of PUMP-1.

By a "substantially pure protein" is meant a protein which has been separated from at least some of those components which naturally accompany it. Typically, the protein is substantially pure when it is at least 60% (by weight) free from the proteins and other naturally-occurring organic molecules

with which it is naturally associated in *vivo*. Preferably, the purity of the preparation (by weight) is at least 75%, more preferably at least 90%, and most preferably at least 99%. A substantially pure PUMP-1 protein may be obtained, for example, by extraction from a natural source; by expression of a recombinant nucleic acid encoding a PUMP-1 polypeptide; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, *e.g.*, column chromatography, such as immunoaffinity chromatography using an antibody specific for PUMP-1, polyacrylamide gel electrophoresis, or HPLC analysis. A protein is substantially free of naturally associated components when it is separated from at least some of those contaminants which accompany it in its natural state. Thus, a protein which is chemically synthesized or produced in a cellular system different from the cell from which it naturally originates will be, by definition, substantially free from its naturally associated components. Accordingly, substantially pure proteins include eukaryotic proteins synthesized in *E. coli*, other prokaryotes, or any other organism in which they do not naturally occur.

The term "oligonucleotide", as used herein, is defined as a molecule comprised of two or more ribonucleotides, preferably more than three. Its exact size will depend upon many factors, which, in turn, depend upon the ultimate function and use of the oligonucleotide. The term "primer", as used herein, refers to an oligonucleotide, whether occurring naturally (as in a purified restriction digest) or produced synthetically, and which is capable of initiating synthesis of a strand complementary to a nucleic acid when placed under appropriate conditions, *i.e.*, in

the presence of nucleotides and an inducing agent, such as a DNA polymerase, and at a suitable temperature and pH. The primer may be either single-stranded or double-stranded and must be sufficiently long to prime the synthesis of the desired extension product in the presence of the inducing agent. The exact length of the primer will depend upon many factors, including temperature, sequence and/or homology of primer and the method used. For example, in diagnostic applications, the oligonucleotide primer typically contains 15-25 or more nucleotides, depending upon the complexity of the target sequence, although it may contain fewer nucleotides.

The primers herein are selected to be "substantially" complementary to particular target DNA sequences. This means that the primers must be sufficiently complementary to hybridize with their respective strands. Therefore, the primer sequence need not reflect the exact sequence of the template. For example, a non-complementary nucleotide fragment (*i.e.*, containing a restriction site) may be attached to the 5' end of the primer, with the remainder of the primer sequence being complementary to the strand. Alternatively, non-complementary bases or longer sequences can be interspersed into the primer, provided that the primer sequence has sufficient complementary with the sequence to hybridize therewith and form the template for synthesis of the extension product.

The probe to which the DNA of the invention hybridizes preferably consists of a sequence of at least 20 consecutive nucleotides, more preferably 40 nucleotides, even more preferably 50 nucleotides, and most preferably 100

nucleotides or more (up to 100%) of the coding sequence of the nucleotides listed in SEQ ID No. 29 or the complement thereof. Such a probe is useful for detecting expression of PUMP-1 in a cell by a method including the steps of (a) contacting mRNA obtained from the cell with a labeled PUMP-1 hybridization probe; and (b) detecting hybridization of the probe with the mRNA.

By "high stringency" is meant DNA hybridization and wash conditions characterized by high temperature and low salt concentration, *e.g.*, wash conditions of 65°C at a salt concentration of approximately 0.1X SSC, or the functional equivalent thereof. For example, high stringency conditions may include hybridization at about 42°C in the presence of about 50% formamide; a first wash at about 65°C with about 2X SSC containing 1% SDS; followed by a second wash at about 65°C with about 0.1X SSC.

The DNA may have at least about 70% sequence identity to the coding sequence of the nucleotides listed in SEQ ID No. 28, preferably at least 75% (*e.g.*, at least 80%); and most preferably at least 90%. The identity between two sequences is a direct function of the number of matching or identical positions. When a position in both of the two sequences is occupied by the same monomeric subunit, *e.g.*, if a given position is occupied by an adenine in each of two DNA molecules, then they are identical at that position. For example, if 7 positions in a sequence 10 nucleotides in length are identical to the corresponding positions in a second 10-nucleotide sequence, then the two sequences have 70% sequence identity. The length of comparison sequences will generally be at least 50 nucleotides, preferably at

least 60 nucleotides, more preferably at least 75 nucleotides, and most preferably 100 nucleotides. Sequence identity is typically measured using sequence analysis software (*e.g.*, Sequence Analysis Software Package of the Genetics Computer Group (GCG), University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705).

The present invention comprises a vector comprising a DNA sequence which encodes a human PUMP-1 protein, wherein said vector is capable of replication in a host, and comprises, in operable linkage: a) an origin of replication; b) a promoter; and c) a DNA sequence coding for said PUMP-1 protein. Preferably, the vector of the present invention contains a portion of the DNA sequence shown in SEQ ID No. 29. Vectors may be used to amplify and/or express nucleic acid encoding a PUMP-1 protein or fragment thereof.

In addition to substantially full-length proteins, the invention also includes fragments (*e.g.*, antigenic fragments) of the PUMP-1 protein. As used herein, "fragment," as applied to a polypeptide, will ordinarily be at least 10 residues, more typically at least 20 residues, and preferably at least 30 (*e.g.*, 50) residues in length, but less than the entire, intact sequence. Fragments of the PUMP-1 protein can be generated by methods known to those skilled in the art, *e.g.*, by enzymatic digestion of naturally occurring or recombinant PUMP-1 protein, by recombinant DNA techniques using an expression vector that encodes a defined fragment of PUMP-1, or by chemical synthesis. The ability of a candidate fragment to exhibit a characteristic of PUMP-1 (*e.g.*, binding to an antibody specific for PUMP-1) can be

assessed by methods described herein. Purified PUMP-1 or antigenic fragments of PUMP-1 can be used to generate new antibodies or to test existing antibodies (*e.g.*, as positive controls in a diagnostic assay) by employing standard protocols known to those skilled in the art. Included in this invention is polyclonal antisera generated by using PUMP-1 or a fragment of PUMP-1 as the immunogen in, *e.g.*, rabbits. Standard protocols for monoclonal and polyclonal antibody production known to those skilled in this art are employed. The monoclonal antibodies generated by this procedure can be screened for the ability to identify recombinant PUMP-1 cDNA clones, and to distinguish them from other cDNA clones.

Further included in this invention are PUMP-1 proteins which are encoded, at least in part, by portions of SEQ ID No. 29, *e.g.*, products of alternative mRNA splicing or alternative protein processing events, or in which a section of PUMP-1 sequence has been deleted. The fragment, or the intact PUMP-1 polypeptide, may be covalently linked to another polypeptide, *e.g.*, one which acts as a label, a ligand or a means to increase antigenicity.

The invention also includes a polyclonal or monoclonal antibody which specifically binds to PUMP-1. The invention encompasses not only an intact monoclonal antibody, but also an immunologically-active antibody fragment, *e.g.*, a Fab or $(Fab)_2$ fragment; an engineered single chain Fv molecule; or a chimeric molecule, *e.g.*, an antibody which contains the binding specificity of one antibody, *e.g.*, of murine origin, and the remaining portions of another antibody, *e.g.*, of human origin.

In one embodiment, the antibody, or a fragment

thereof, may be linked to a toxin or to a detectable label, *e.g.*, a radioactive label, non-radioactive isotopic label, fluorescent label, chemiluminescent label, paramagnetic label, enzyme label, or colorimetric label. Examples of suitable toxins include diphtheria toxin, *Pseudomonas* exotoxin A, ricin, and cholera toxin. Examples of suitable enzyme labels include malate hydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, alcohol dehydrogenase, alpha-glycerol phosphate dehydrogenase, triose phosphate isomerase, peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, acetylcholinesterase, etc. Examples of suitable radioisotopic labels include ^{3}H, ^{125}I, ^{131}I, ^{32}P, ^{35}S, ^{14}C, etc.

Paramagnetic isotopes for purposes of *in vivo* diagnosis can also be used according to the methods of this invention. There are numerous examples of elements that are useful in magnetic resonance imaging. For discussions on *in vivo* nuclear magnetic resonance imaging, see, for example, Schaefer et al., (1989) *JACC* 14, 472-480; Shreve et al., (1986) *Magn. Reson. Med.* 3, 336-340; Wolf, G. L., (1984) *Physiol. Chem. Phys. Med. NMR* 16, 93-95; Wesbey et al., (1984) *Physiol. Chem. Phys. Med. NMR* 16, 145-155; Runge et al., (1984) *Invest. Radiol.* 19, 408-415. Examples of suitable fluorescent labels include a fluorescein label, an isothiocyalate label, a rhodamine label, a phycoerythrin label, a phycocyanin label, an allophycocyanin label, an ophthaldehyde label, a fluorescamine label, etc. Examples of chemiluminescent labels include a luminal label, an isoluminal label, an aromatic acridinium ester label, an imidazole

label, an acridinium salt label, an oxalate ester label, a luciferin label, a luciferase label, an aequorin label, etc.

Those of ordinary skill in the art will know of other suitable labels which may be employed in accordance with the present invention. The binding of these labels to antibodies or fragments thereof can be accomplished using standard techniques commonly known and used by those of ordinary skill in the art. Typical techniques are described by Kennedy et al., (1976) *Clin. Chim. Acta* 70, 1-31; and Schurs et al., (1977) *Clin. Chim. Acta* 81, 1-40. Coupling techniques mentioned in the latter are the glutaraldehyde method, the periodate method, the dimaleimide method, the m-maleimidobenzyl-N-hydroxy-succinimide ester method. All of these methods are incorporated by reference herein.

Also within the invention is a method of detecting PUMP-1 protein in a biological sample, which includes the steps of contacting the sample with the labeled antibody, *e.g.*, radioactively tagged antibody specific for PUMP-1, and determining whether the antibody binds to a component of the sample. Antibodies to the PUMP-1 protein can be used in an immunoassay to detect increased levels of PUMP-1 protein expression in tissues suspected of neoplastic transformation. These same uses can be achieved with Northern blot assays and analyses.

As described herein, the invention provides a number of diagnostic advantages and uses. For example, the PUMP-1 protein is useful in diagnosing cancer in different tissues since this protein is highly overexpressed in tumor cells. Antibodies

(or antigen-binding fragments thereof) which bind to an epitope specific for PUMP-1 are useful in a method of detecting PUMP-1 protein in a biological sample for diagnosis of cancerous or neoplastic transformation. This method includes the steps of obtaining a biological sample (*e.g.*, cells, blood, plasma, tissue, etc.) from a patient suspected of having cancer, contacting the sample with a labeled antibody (*e.g.*, radioactively tagged antibody) specific for PUMP-1, and detecting the PUMP-1 protein using standard immunoassay techniques such as an ELISA. Antibody binding to the biological sample indicates that the sample contains a component which specifically binds to an epitope within PUMP-1.

Likewise, a standard Northern blot assay can be used to ascertain the relative amounts of PUMP-1 mRNA in a cell or tissue obtained from a patient suspected of having cancer, in accordance with conventional Northern hybridization techniques known to those of ordinary skill in the art. This Northern assay uses a hybridization probe, *e.g.*, radiolabelled PUMP-1 cDNA, either containing the full-length, single stranded DNA having a sequence complementary to SEQ ID No. 29, or a fragment of that DNA sequence at least 20 (preferably at least 30, more preferably at least 50, and most preferably at least 100 consecutive nucleotides in length). The DNA hybridization probe can be labeled by any of the many different methods known to those skilled in this art.

The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion:

EXAMPLE 1

Amplification of serine proteases using redundant and specific primers

Only cDNA preparations deemed free of genomic DNA were used for gene expression analysis. Redundant primers were prepared for serine proteases, metallo-proteases and cysteine protease. The primers were synthesized to consensus sequences of amino acid surrounding the catalytic triad for serine proteases, *viz.* histidine ... aspartate ... and serine. The sequences of both sense (histidine & aspartate) and antisense (aspartate and serine) redundant primers are shown in Table 2.

TABLE 2

PCR Primers

	5'→3'	SEQ ID No.
Redundant Primers:		
Serine Protease (histidine) = S1	tgggtigtiacigcigcica(ct)t	1
Serine Protease (aspartic acid) = AS1	a(ag)ia(ag)igciatitcitticc	2
Serine Protease (serine) = AS11	a(ag)iggiccicci(cg)(ta)(ag)tcicc	3
Cysteine Protease - sense	ca(ag)ggica(ag)tg(ct)ggi(ta)(cg)itg(ct)tgg	4
Cysteine Protease - antisense	taiccicc(ag)tt(ag)caicc(ct)tc	5
Metallo Protease - sense	cci(ac)gitg(tc)ggi(ga)(ta)icciga	6
Metallo Protease - antisense	tt(ag)tgicciai(ct)tc(ag)tg	7
Specific Primers:		
Serine Protease (hepsin) = sense	tgtcccgatggcgagtgttt	8
Serine Protease (hepsin) = antisense	cctgttggccatagtactgc	9
Serine Protease (SCCE) = sense	agatgaatgagtacaccgtg	10
Serine Protease (SCCE) = antisense	ccagtaagtccttgtaaacc	11
Serine Protease (Comp B) = sense	aagggacacgagagctgtat	12
Serine Protease (Comp B) = antisense	aagtggtagttggaggaagc	13
Serine Protease (Protease M)= sense	ctgtgatccaccctgactat	20
Serine Protease (Protease M) = antisense	caggtggatgtatgcacact	21
Serine Protease (TADG12) = sense (Ser10-s)	gcgcactgtgtttatgagat	22
Serine Protease (TADG12) = antisense (Ser10-as)	ctctttggcttgtacttgct	23
Serine Protease (TADG13) = sense	tgagggacatcattatgcac	24
Serine Protease (TADG13) = antisense	caagttttccccataattgg	25
Serine Protease (TADG14) = sense	acagtacgcctgggagacca	26
Serine Protease (TADG14) = antisense	ctgagacggtgcaattctgg	27
Cysteine Protease (Cath-L) = sense	attggagagagaaaggctac	14
Cysteine Protease (Cath-L) = antisense	cttgggattgtacttacagg	15
Metallo Protease (PUMP1) = sense	cttccaaagtggtcacctac	16
Metallo Protease (PUMP1) = antisense	ctagactgctaccatccgtc	17

EXAMPLE 2

Carcinoma tissue

Several protease entities were identified and

subcloned from PCR amplification of cDNA derived from serous cystadenocarcinomas. Therefore, the proteases described herein are reflective of surface activities for this type of carcinoma, the most common form of ovarian cancer. Applicant has also shown PCR amplification bands unique to the mucinous tumor type and the clear cell type of similar base pair size. About 20-25% of ovarian cancers are classified as either mucinous, clear cell, or endometrioid.

EXAMPLE 3

Ligation, transformation and sequencing

To determine the identity of the PCR products, all the appropriate bands were ligated into Promega T-vector plasmid and the ligation product was used to transform JM109 cells (Promega) grown on selective media. After selection and culturing of individual colonies, plasmid DNA was isolated by means of the WIZARD MINIPREP™ DNA purification system (Promega). Inserts were sequenced using a Prism Ready Reaction Dydeoxy Terminators cycle sequencing kit (Applied Biosystems). Residual dye terminators were removed from the completed sequencing reaction using a CENTRISEP SPIN™ column (Princeton Separation), and samples were loaded into an Applied Biosystems Model 373A DNA sequencing system. The results of subcloning and sequencing for the serine protease primers are summarized in Table 3.

TABLE 3

Serine protease candidates

Subclone	Primer Set	Gene Candidate
1	His-Ser	hepsin
2	His-Ser	SCCE
3	His-Ser	Compliment B
4	His-Asp	Cofactor 1
5	His-Asp	TADG-12*
6	His-Ser	TADG-13*
7	His-Ser	TADG-14*
8	His-Ser	Protease M
9	His-Ser	TADG-15*

*indicates novel proteases

EXAMPLE 4

Cloning and characterization

Cloning and characterization of new gene candidates was undertaken to expand the panel representative of extracellular proteases specific for ovarian carcinoma subtypes. Sequencing of the PCR products derived from tumor cDNA confirms the potential candidacy of these genes. The three novel genes all have conserved residues within the catalytic triad sequence consistent with their membership in the serine protease family. Applicant compared the PCR products amplified from normal and carcinoma cDNAs using sense-histidine and antisense-aspartate as well as sense-histidine and antisense-serine. The anticipated PCR products of approximately 200 bp and 500 bp for those pairs of primers were observed (aspartate is approximately

50-70 amino acids downstream from histidine, and serine is about 100-150 amino acids toward the carboxy end from histidine).

Figure 1 shows a comparison of PCR products derived from normal and carcinoma cDNA as shown by staining in an agarose gel. Two distinct bands in lane 2 were present in the primer pair sense-His/antisense ASP (AS1) and multiple bands of about 500 bp are noted in the carcinoma lane for the sense-His/antisense-Ser (AS2) primer pairs in lane 4.

EXAMPLE 5

Quantitative PCR

The mRNA overexpression of PUMP-1 was detected and determined using quantitative PCR. Quantitative PCR was performed generally according to the method of Noonan et al. [*Proc.Natl.Acad.Sci.,USA*, 87:7160-7164 (1990)]. The following oligonucleotide primers were used:

PUMP-1:

forward 5'-CTTCCAAAGTGGTCACCTAC-3' (SEQ ID No. 16), and

reverse 5'-CTAGACTGCTACCATCCGTC-3' (SEQ ID No. 17);

and β-tubulin:

forward 5'- TGCATTGACAACGAGGC -3' (SEQ ID No. 18), and

reverse 5'- CTGTCTTGA CATTGTTG -3' (SEQ ID No. 19).

β-tubulin was utilized as an internal control. The predicted sizes of the amplified genes were 250 bp for PUMP-1 and 454 bp for β-tubulin. The primer sequences used in this study were designed according to the cDNA sequences described by Leytus *et al.*

[*Biochemistry*, *27*, 1067-1074 (1988)] for PUMP-1, and Hall *et al.* [*Mol. Cell. Biol.*, *3*, 854-862 (1983)] for β-tubulin. The PCR reaction mixture consisted of cDNA derived from 50 ng of mRNA converted by conventional techniques, 5 pmol of sense and antisense primers for both the PUMP-1 gene and the β-tubulin gene, 200 μmol of dNTPs, 5 μCi of α-^{32}PdCTP and 0.25 units of Taq DNA polymerase with reaction buffer (Promega) in a final volume of 25 μl. The target sequences were amplified in parallel with the β-tubulin gene. Thirty cycles of PCR were carried out in a Thermal Cycler (Perkin-Elmer Cetus). Each cycle of PCR included 30 sec of denaturation at 95°C, 30 sec of annealing at 63°C and 30 sec of extension at 72°C. The PCR products were separated on 2% agarose gels and the radioactivity of each PCR product was determined by using a PhosphorImager™ (Molecular Dynamics). Student's *t* test was used for comparison of mean values.

Experiments comparing PCR amplification in normal ovary and ovarian carcinoma suggested overexpression and/or alteration in mRNA transcript in tumor tissues. Northern blot analysis of TADG-14 confirms a transcript size of 1.4 kb and data indicate overexpression in ovarian carcinoma (Figure 2). Isolation and purification using both PCR and a specific 250 bp PCR product to screen positive plaques yielded a 1.2 kb clone of TADG-14. Other proteases were amplified by the same method using the appropriate primers from Table 2.

EXAMPLE 6

Tissue bank

A tumor tissue bank of fresh frozen tissue of ovarian

carcinomas as shown in Table 4 was used for evaluation. Approximately 100 normal ovaries removed for medical reasons other than malignancy were obtained from surgery and were available as controls.

TABLE 4

Ovarian cancer tissue bank

	Total	Stage I/II	Stage III/IV	No Stage
Serous				
Malignant	166	15	140	8
LMP	16	9	7	0
Benign	12	0	0	12
Mucinous				
Malignant	26	6	14	6
LMP	28	25	3	0
Benign	3	0	0	3
Endometrioid				
Malignant	38	17	21	0
LMP	2	2	0	0
Benign	0	0	0	0
Other*				
Malignant	61	23	29	9
LMP	0	0	0	0
Benign	5	0	0	5

*Other category includes the following tumor types: Brenner's tumor, thecoma, teratoma, fibrothecoma, fibroma, granulosa cell, clear cell, germ cell, mixed mullerian, stromal, undifferentiated, and dysgerminoma.

From the tumor bank, approximately 100 carcinomas were evaluated encompassing most histological sub-types of ovarian carcinoma, including borderline or low-malignant potential tumors and overt carcinomas. The approach included using mRNA prepared from fresh frozen tissue (both normal and malignant) to compare expression of genes in normal, low malignant potential tumors and overt carcinomas. The cDNA prepared from polyA$^+$ mRNA was deemed to be genomic DNA-free by checking all preparations with primers that encompassed a known intron-exon splice site using both β-tubulin and p53 primers.

EXAMPLE 7

Northern blots

Significant information can be obtained by examining the expression of these candidate genes by Northern blot. Analysis of normal adult multi-tissue blots offers the opportunity to identify normal tissues which may express the protease. Ultimately, if strategies for inhibition of proteases for therapeutic intervention are to be developed, it is essential to appreciate the expression of these genes in normal tissue if and when it occurs.

Significant information is expected from Northern blot analysis of fetal tissue. Genes overexpressed in carcinomas are often highly expressed in organogenesis. As indicated, the hepsin gene cloned from hepatoma cells and overexpressed in ovarian carcinoma is overtly expressed in fetal liver. Hepsin gene expression was also detected in fetal kidney, and therefore, could

be a candidate for expression in renal carcinomas.

Northern panels for examining expression of genes in a multi-tissue normal adult as well as fetal tissue are commercially available (CLONTECH). Such evaluation tools are not only important to confirm the overexpression of individual transcripts in tumor versus normal tissues, but also provides the opportunity to confirm transcript size, and to determine if alternate splicing or other transcript alteration may occur in ovarian carcinoma.

EXAMPLE 8

Northern blot analysis

Northern blot analysis was performed as follows: 10 µg of mRNA was loaded onto a 1% formaldehyde-agarose gel, electrophoresed and blotted onto a HyBond-N+™ nylon membrane (Amersham). ^{32}P-labeled cDNA probes were made using Prime-a-Gene Labeling System™ (Promega). The PCR products amplified by specific primers were used as probes. Blots were prehybridized for 30 min and then hybridized for 60 min at 68°C with ^{32}P-labeled cDNA probe in ExpressHyb™ Hybridization Solution (CLONTECH). Control hybridization to determine relative gel loading was accomplished using the β-tubulin probe.

EXAMPLE 9

PCR products corresponding to serine, cysteine and metallo-proteases

Based on their unique expression in either low

malignant potential tumors or carcinomas, PCR-amplified cDNA products were cloned and sequenced and the appropriate gene identified based upon nucleotide and amino acid sequences stored in the GCG and EST databases. Figures 3, 4 & 5 show the PCR product displays comparing normal and carcinomatous tissues using redundant primers for serine proteases (Figure 3), for cysteine proteases (Figure 4) and for metallo-proteases (Figure 5). Note the differential expression in the carcinoma tissues versus the normal tissues. The proteases were identified using redundant cDNA primers (see Table 2) directed towards conserved sequences that are associated with intrinsic enzyme activity (for serine proteases, cysteine proteases and metallo-proteases) by comparing mRNA expression in normal, low malignant potential and overt ovarian carcinoma tissues according to Sakanari *et al.* [*Biochemistry* *86*, 4863-4867 (1989)].

EXAMPLE 10

Serine proteases

For the serine protease group, using the histidine domain primer sense, S1, in combination with antisense primer AS2, the following proteases were identified:

(a) Hepsin, a trypsin-like serine protease cloned from hepatoma cells shown to be a cell surface protease essential for the growth of hepatoma cells in culture and highly expressed in hepatoma tumor cells (Figure 3, lane 4);

(b) Complement factor B protease (human factor IX), a protease involved in the coagulation cascade and associated with

the production and accumulation of fibrin split products associated with tumor cells (Figure 3, lane 4). Compliment factor B belongs in the family of coagulation factors X (Christmas factor). As part of the intrinsic pathway, compliment factor B catalyzes the proteolytic activation of coagulation factor X in the presence of Ca^{2+} phospholipid and factor VIIIa e5; and

(c) A stratum corneum chymotryptic enzyme (SCCE) serine protease involved in desquarnation of skin cells from the human stratum corneum (Figure 3, lane 4). SCCE is expressed in keratinocytes of the epidermis and functions to degrade the cohesive structures in the cornified layer to allow continuous skin surface shedding.

EXAMPLE 11

Cysteine proteases

In the cysteine protease group, using redundant sense and anti-sense primers for cysteine proteases, one unique PCR product was identified by overexpression in ovarian carcinoma when compared to normal ovarian tissue (Figure 4, lanes 3-5). Cloning and sequencing this PCR product identified a sequence of Cathepsin L, which is a lysomal cysteine protease whose expression and secretion is induced by malignant transformation, growth factors and tumor promoters. Many human tumors (including ovarian) express high levels of Cathepsin L. Cathepsin L cysteine protease belongs in the stromolysin family and has potent elastase and collagenase activities. Published data indicates increased levels in the serum of patients with mucinous

cystadenocarcinoma of the ovary. It has not heretofore been shown to be expressed in other ovarian tumors.

EXAMPLE 12

Metallo-proteases

Using redundant sense and anti-sense primers for the metallo-protease group, one unique PCR product was detected in the tumor tissue which was absent in normal ovarian tissue (Figure 5, lanes 2-5). Subcloning and sequencing this product indicates it has complete homology in the appropriate region with the so-called PUMP-1 (MMP-7) gene. This zinc-binding metallo-protease is expressed as a proenzyme with a signal sequence and is active in gelatin and collagenase digestion. PUMP-1 has also been shown to be induced and overexpressed in 9 of 10 colorectal carcinomas compared to normal colon tissue, suggesting a role for this substrate in the progression of this disease.

EXAMPLE 13

Expression of PUMP-1

The expression of the metallo-protease PUMP-1 gene in 10 normal ovaries, 12 low malignant potential (LMP) tumors, and 32 ovarian carcinoma (both mucinous and serous type) by quantitative PCR using PUMP-1-specific primers (see Table 2) was determined (primers directed toward the β-tubulin message were used as an internal standard) (Table 5). Using a cut-off level for overexpression of the mean for normal ovary + 2SD, 9 of 12 LMP (75%) tumor cases and 26 of 32 (81%) carcinoma cases were

above the cut-off value. PUMP-1 mRNA expression was significantly elevated in tumors compared to that in normal ovary for both LMP tumor ($p<0.05$) and carcinoma ($p<0.0001$). All 10 cases of normal ovaries showed relatively low levels of PUMP-1 mRNA expression.

Table 5 summarizes the data obtained on the histological type, stage, grade and mRNA overexpression of PUMP-1 in all the cases studied. Lymph node metastases were histopathologically proven in 5 cases and all these cases showed overexpression of PUMP-1. Also of note, all 5 cases of stage I carcinoma showed overexpression of PUMP-1. Overall, the expression ratio (mean±SD) for normal ovary was determined to be 0.084±0.065; for LMP tumors, 0.905±1.251; and for carcinomas, 0.663±0.630 (Table 6). From a histological point of view (Table 6), overexpression of PUMP-1 was observed in 21 of 26 (80.8%) serous tumors (6 of 7 LMP tumors and 15 of 19 carcinomas) and 8 of 12 (66.7%) mucinous tumors (3 of 5 LMP tumors and 5 of 7 carcinomas). For endometrioid and clear cell carcinomas, PUMP-1 was found to be overexpressed in all 6 cases examined.

TABLE 5

Patient Characteristics and Expression of PUMP-1 Gene

Case	Histological type[a]	Stage/Grade	LN[b]	mRNA expression
1	normal ovary			n
2	normal ovary			n
3	normal ovary			n

4	normal ovary			n
5	normal ovary			n
6	normal ovary			n
7	normal ovary			n
8	normal ovary			n
9	normal ovary			n
10	normal ovary			n
11	S adenoma (LMP)	1/1	N	4+
12	S adenoma (LMP)	1/1	NE	n
13	S adenoma (LMP)	1/1	NE	4+
14	S adenoma (LMP)	1/1	N	4+
15	S adenoma (LMP)	3/1	P	4+
16	S adenoma (LMP)	3/1	P	4+
17	S adenoma (LMP)	3/1	P	4+
18	M adenoma (LMP)	1/1	NE	n
19	M adenoma (LMP)	1/1	N	n
20	M adenoma (LMP)	1/1	N	4+
21	M adenoma (LMP)	1/1	NE	4+
22	M adenoma (LMP)	1/1	NE	4+
23	S carcinoma	1/2	N	4+
24	S carcinoma	1/3	N	4+
25	S carcinoma	3/1	NE	4+
26	S carcinoma	3/2	NE	4+
27	S carcinoma	3/2	P	4+
28	S carcinoma	3/2	NE	2+
29	S carcinoma	3/3	NE	n
30	S carcinoma	3/3	NE	4+
31	S carcinoma	3/3	NE	4+

32	S carcinoma	3/3	NE	4+
33	S carcinoma	3/3	N	2+
34	S carcinoma	3/3	NE	n
35	S carcinoma	3/3	NE	4+
36	S carcinoma	3/3	NE	4+
37	S carcinoma	3/3	NE	2+
38	S carcinoma	3/3	N	n
39	S carcinoma	3/2	NE	4+
40	S carcinoma	3/3	NE	2+
41	S carcinoma	3/2	NE	n
42	M carcinoma	1/2	N	4+
43	M carcinoma	2/2	NE	4+
44	M carcinoma	2/2	N	4+
45	M carcinoma	3/1	NE	4+
46	M carcinoma	3/2	NE	n
47	M carcinoma	3/2	NE	n
48	M carcinoma	3/3	NE	4+
49	E carcinoma	2/3	N	4+
50	E carcinoma	3/2	NE	4+
51	E carcinoma	3/3	NE	4+
52	C carcinoma	1/3	N	4+
53	C carcinoma	1/1	N	4+
54	C carcinoma	3/2	P	4+

[a]S, serous; M, mucinous; E, endometrioid; C, clear cell; [b]LN, lymph node metastasis; P, positive; N, negative; NE, not examined; [c]n, normal range = mean ±2SD; 2+, mean +2SD to +4SD; 4+, mean +4SD or greater.

TABLE 6

Overexpression of PUMP-1 in normal ovaries and ovarian tumors

Type	N	PUMP-1 Overexpression	Ratio of PUMP-1 to β-tubulin
Normal	10	0 (0%)	0.084 ± 0.065
LMP	12	9 (75.0%)	0.905 ± 1.251
Serous	7	6 (85.7%)	1.301 ± 1.542
Mucinous	5	3 (60.0%)	0.351 ± 0.269
Carcinomous	32	26 (81.3%)	0.663 ± 0.630
Serous	19	15 (78.9%)	0.675 ± 0.774
Mucinous	7	5 (71.4%)	0.474 ± 0.337
Endometrioid	3	3 (100%)	0.635 ± 0.224
Clear Cell	3	3 (100%)	1.062 ± 0.060

EXAMPLE 14

Expression of SCCE and PUMP-1

Studies using both SCCE-specific primers (Figure 8) and PUMP-specific primers (Figure 9) indicate overexpression of these proteases in ovarian carcinomas.

Examination of PUMP-1 antigen expression in normal and carcinomatous tissues by immunolocalization using both a peptide derived polyclonal antibody and a commercial monoclonal antibody (Calbiochem) confirmed the quantitative PCR data shown in Table 7. Little or no staining was observed in normal ovary (Figure 20A), while intense tumor cell staining of

secretory bodies could be detected in several mucinous tumors (*e.g.*, Figure 20B). Intense cytoplasmic staining was also observed in serous tumors (Figure 20C), clear cell tumors (Figure 20D) and a secreted product was most noticeable in endometrioid tumors (Figure 20E).

TABLE 7

Expression of PUMP-1 protein by immunolocalization

Histology	mRNA[a]	Protein[b]
Normal ovary	n	-
S Carcinoma	4+	+
S Carcinoma	4+	+
S Carcinoma	4+	+
S Carcinoma	4+	+
S Carcinoma	2+	+
S Carcinoma	n	-
S Carcinoma	4+	+
S Carcinoma	2+	+
S Carcinoma	n	-
M Carcinoma	n	-
C Carcinoma	4+	+
C Carcinoma	4+	+
C Carcinoma	4+	+

[a]mRNA expression of PUMP-1 (see Table 5). n = low or no transcript detected by quantitative PCR. 2+/4+ = overexpression of PUMP-1 transcription by more than 2SD or 4SD over the normal level of PUMP-1 in normal ovary. [b] +, >10% positive tumor cells; -, negative.

EXAMPLE 15

Summary of known proteases detected herein

Most of the proteases described herein were identified from the sense-His/antisense-Ser primer pair, yielding a 500 bp PCR product (Figure 1, lane 4). Some of the enzymes are familiar, a short summary of each follows.

Hepsin

Hepsin is a trypsin-like serine protease cloned from hepatoma cells. Hepsin is an extracellular protease (the enzyme includes a secretion signal sequence) which is anchored in the plasma membrane by its amino terminal domain, thereby exposing its catalytic domain to the extracellular matrix. Hepsin has also been shown to be expressed in breast cancer cell lines and peripheral nerve cells. It has never before been associated with ovarian carcinoma. Specific primers for the hepsin gene were synthesized and the expression of hepsin examined using Northern blots of fetal tissue and ovarian tissue (both normal and ovarian carcinoma).

Figure 10A shows that hepsin was expressed in fetal liver and fetal kidney as anticipated, but at very low levels or not at all in fetal brain and lung. Figure 10B shows that hepsin was expressed in ovarian carcinomas of different histologic types, but not in normal ovary. The mRNA identified in both Northern blots was the appropriate size for the hepsin transcript. The expression of hepsin was examined in 10 normal ovaries and 44 ovarian tumors using specific primers to β-tubulin and hepsin in a quantitative PCR assay, and found it to be linear over 35 cycles. Expression is presented as the ratio of ^{32}P-hepsin band to the

internal control, the ^{32}P-β-tubulin band.

Figures 11A & 11B show hepsin expression in normal (N), mucinous (M) and serous (S) low malignant potential (LMP) tumors and carcinomas (CA). Figure 11A shows quantitative PCR of hepsin and internal control β-tubulin. Figure 11B shows a bar graph of expression of PUMP-1 in 10 normal ovaries and 44 ovarian carcinoma samples.

Hepsin mRNA is highly overexpressed in most histopathologic types of ovarian carcinomas including some low malignant potential tumors (see Figures 11A & 11B). Most noticeably, hepsin is highly expressed in serous, endometrioid and clear cell tumors tested. It is highly expressed in some mucinous tumors, but it is not overexpressed in the majority of such tumors.

Stratum corneum chymotrypsin enzyme (SCCE)

The PCR product identified was the catalytic domain of the sense-His/antisense-Ser of the SCCE enzyme. This extracellular protease was cloned, sequenced and shown to be expressed on the surface of keratinocytes in the epidermis. SCCE is a chymotrypsin-like serine protease whose function is suggested to be in the catalytic degradation of intercellular cohesive structures in the stratum corneum layer of the skin. This degradation allows continuous shedding (desquamation) of cells from the skin surface. The subcellular localization of SCCE is in the upper granular layer in the stratum corneum of normal non-palmoplantar skin and in the cohesive parts of hypertrophic plantar stratum corneum. SCCE is exclusively associated with the stratum corneum and has not so far been shown to be expressed

in any carcinomatous tissues.

Northern blots were probed with the PCR product to determine expression of SCCE in fetal tissue and ovarian carcinoma (Figures 12A & 12B). Noticeably, detection of SCCE messenger RNA on the fetal Northern was almost non-existent (a problem with the probe or the blot was excluded by performing the proper controls). A faint band appeared in fetal kidney. On the other hand, SCCE mRNA is abundant in the ovarian carcinoma mRNA (Figure 12B). Two transcripts of the correct size are observed for SCCE. The same panel of cDNA used for hepsin analysis was used for SCCE expression.

No SCCE expression was detected in the normal ovary lane of the Northern blot. A comparison of all candidate genes, including a loading marker (β-tubulin), was shown to confirm that this observation was not a result of a loading bias. Quantitative PCR using SCCE primers, along with β-tubulin internal control primers, confirmed the overexpression of SCCE mRNA in carcinoma of the ovary with no expression in normal ovarian tissue (Figure 13).

Figure 13A shows a comparison using quantitative PCR of SCCE cDNA from normal ovary and ovarian carcinomas. Figure 13B shows the ratio of SCCE to the β-tubulin internal standard in 10 normal and 44 ovarian carcinoma tissues. Again, it is observed that SCCE is highly overexpressed in ovarian carcinoma cells. It is also noted that some mucinous tumors overexpress SCCE, but the majority do not.

Protease M

Protease M was identified from subclones of the His--

ser primer pair. This protease was first cloned by Anisowicz, *et al.*, [*Molecular Medicine,* 2, 624-636 (1996)] and shown to be overexpressed in breast and ovarian carcinomas. A preliminary evaluation indicates that this enzyme is overexpressed in ovarian carcinoma (Figure 14).

Cofactor I and Complement factor B

Several serine proteases associated with the coagulation pathway were also subcloned. Examination of normal and ovarian carcinomas by quantitative PCR for expression of these enzymes, it was noticeable that this mRNA was not clearly overexpressed in ovarian carcinomas when compared to normal ovarian tissue. It should be noted that the same panel of tumors was used for the evaluation of each candidate protease.

EXAMPLE 16

Summary of previously unknown proteases detected herein

TADG-12

TADG-12 was identified from the primer pairs, sense-His/antisense-Asp (see Figure 1, lanes 1 & 2). Upon subcloning both PCR products in lane 2, the 200 bp product had a unique protease-like sequence not included in GenBank. This 200 bp product contains many of the conserved amino acids common for the His-Asp domain of the family of serine proteins. The second and larger PCR product (300 bp) was shown to have a high degree of homology with TADG-12 (His-Asp sequence), but also contained approximately 100 bp of unique sequence. Synthesis of specific primers and the sequencing of the subsequent PCR

products from three different tumors demonstrated that the larger PCR product (present in about 50% of ovarian carcinomas) includes an insert of about 100 bp near the 5' end (and near the histidine) of the sequence. This insert may be a retained genomic intron because of the appropriate position of splice sites and the fact that the insert does not contain an open reading frame (see Figure 15). This suggests the possibility of a splice site mutation which gives rise to retention of the intron, or a translocation of a sequence into the TADG-12 gene in as many as half of all ovarian carcinomas.

TADG-13 and TADG-14

Specific primers were synthesized for TADG-13 and TADG-14 to evaluate expression of genes in normal and ovarian carcinoma tissue. Northern blot analysis of ovarian tissues indicates the transcript for the TADG-14 gene is approximately 1.4 kb and is expressed in ovarian carcinoma tissues (Figure 16A) with no noticeable transcript presence in normal tissue. In quantitative PCR studies using specific primers, increased expression of TADG-14 in ovarian carcinoma tissues was noted compared to a normal ovary (Figure 16B). The presence of a specific PCR product for TADG-14 in both an HeLa library and an ovarian carcinoma library was also confirmed. Several candidate sequences corresponding to TADG-14 have been screened and isolated from the HeLa library.

Clearly from sequence homology, these genes fit into the family of serine proteases. TADG-13 and -14 are, however, heretofore undocumented genes which the specific primers of the invention allow to be evaluated in normal and tumor cells, and

with which the presence or absence of expression of these genes is useful in the diagnosis or treatment selection for specific tumor types.

PUMP-1

In a similar strategy using redundant primers to metal binding domains and conserved histidine domains, a differentially expressed PCR product identical to matrix metallo-protease 7 (MMP-7) was identified, herein called PUMP-1. Using specific primers for PUMP-1, PCR produced a 250 bp product for Northern blot analysis.

To confirm the results of quantitative PCR and to identify the appropriate transcript size for PUMP-1, Northern blot hybridization was performed using representative samples of each histological type of carcinoma. As shown in Figure 17A, Northern blot hybridization with a ^{32}P-labeled PUMP-1 probe revealed an intense band in carcinoma cases and no visible band in normal ovary. The size of the PUMP-1 transcript in carcinoma cases was approximately 1.1 Kb. Among normal human fetal tissues examined, fetal lung and fetal kidney showed increased transcript expression (Figure 17B), on the other hand, PUMP-1 expression was not observed or was expressed at very low levels in normal human adult tissues, including spleen, thymus, prostate, testis, ovary, small intestine, colon and peripheral blood leukocyte (Figure 17C).

Quantitative PCR comparing normal versus ovarian carcinoma expression of the PUMP-1 mRNA indicates that this gene is highly expressed in serous carcinomas, including most low malignant serous tumors, and is, again, expressed to a lesser

extent in mucinous tumors (see Figures 18A & 18B). PUMP-1, however, is so far the protease most frequently found overexpressed in mucinous tumors (See Table 8).

Cathepsin-L

Using redundant cysteine protease primers to conserved domains surrounding individual cysteine and histidine residues, the cathepsin-L protease was identified in several serous carcinomas. An initial examination of the expression of cathepsin L in normal and ovarian tumor tissue indicates that transcripts for the cathepsin-L protease are present in both normal and tumor tissues (Figure 21). However, its presence or absence in combination with other proteases of the present invention permits identification of specific tumor types and treatment choices.

EXAMPLE 17

Summary of Data

Redundant primers to conserved domains of serine, metallo-, and cysteine proteases have yielded a set of genes whose mRNAs are overexpressed in ovarian carcinoma. The genes which are clearly overexpressed include the serine proteases hepsin, SCCE, protease M TADG12, TADG14 and the metallo-protease PUMP-1 (see Figure 21 and Table 8). Northern blot analysis of normal and ovarian carcinoma tissues, summarized in Figure 14, indicated overexpression of hepsin, SCCE, PUMP-1 and TADG-14. A β-tubulin probe to control for loading levels was included.

TABLE 8

Overexpression of proteases in ovarian tumors

Type	N	PUMP-1	SCCE	Pump-1	Prot M
Normal	10	0% (0/10)	0% (0/10)	0% (0/10)	0% (0/10)
LMP	12	58.3% (7/12)	66.7% (8/12)	75.0% (9/12)	75% (9/12)
Serous	7	85.7% (6/7)	85.7% (6/7)	85.7% (6/7)	100% (7/7)
mucinous	5	20.0% (1/5)	40.0% (2/5)	60% (3/5)	40.0%(2/5)
Carcinoma	32	84.4% (27/32)	78.1% (25/32)	81.3% (26/32)	90.6% (29/32)
serous	19	94.7%(18/19)	89.5%(17/19)	78.9% (15/19)	94.7% (18/19)
mucinous	7	42.9%(3/7)	28.6%(2/7)	71.4% (5/7)	85.7% (6/7)
endometr.	3	100% (3/3)	100%(3/3)	100% (3/3)	100% (3/3)
clear cell	3	100% (3/3)	100% (3/3)	100% (3/3)	67.7% (2/3)

EXAMPLE 18

Implications

For the most part, these proteins previously have not been associated with the extracellular matrix of ovarian carcinoma cells. No panel of proteases which might contribute to the growth, shedding, invasion and colony development of metastatic carcinoma has been previously described, including the three new candidate serine proteases which are herein disclosed. The establishment of an extracellular protease panel associated with either malignant growth or malignant potential offers the opportunity for the identification of diagnostic or prognostic markers and for therapeutic intervention through inhibition or down regulation of these proteases.

The availability of the instant gene-specific primers

coding for the appropriate region of tumor specific proteases allows for the amplification of a specific cDNA probe using Northern and Southern analysis, and their use as markers to detect the presence of the cancer in tissue. The probes also allow more extensive evaluation of the expression of the gene in normal ovary versus low malignant potential tumor, as well as both high- and low-stage carcinomas. The evaluation of a panel of fresh frozen tissue from all the carcinoma subtypes (Table 4) allowed the determination of whether a protease is expressed predominantly in early stage disease or within specific carcinoma subtypes. It was also determined whether each genes' expression is confined to a particular stage in tumor progression and/or is associated with metastatic lesions. Detection of specific combinations of proteases is an identifying characteristic of the specific tumor types and yields valuable information for diagnoses and treatment selection. Particular tumor types may be more accurately diagnosed by the characteristic expression pattern of each specific tumor.

EXAMPLE 19

Antisense PUMP-1

PUMP-1 is cloned and expressed in the opposite orientation such that an antisense RNA molecule (SEQ ID No. 28) is produced. For example, the antisense RNA is used to hybridize to the complementary RNA in the cell and thereby inhibit translation of PUMP-1 RNA into protein.

EXAMPLE 20

Peptide ranking

For vaccine or immune stimulation, individual 9-mers to 11-mers of the PUMP-1 protein were examined to rank the binding of individual peptides to the top 8 haplotypes in the general population (Parker et al., (1994)). The computer program used for this analyses can be found at <http://www-bimas.dcrt.nih.gov/molbio/hla_bind/>. Table 9 shows the peptide ranking based upon the predicted half-life of each peptide's binding to a particular HLA allele. A larger half-life indicates a stronger association with that peptide and the particular HLA molecule. The PUMP-1 peptides that strongly bind to an HLA allele are putative immunogens, and are used to innoculate an individual against PUMP-1.

TABLE 9

PUMP-1 peptide ranking

HLA Type & Ranking	Start	Peptide	Predicted Dissociation$_{1/2}$	SEQ ID No.
HLA A0201				
1	208	FLYAATHEL	314.455	30
2	134	NMWGKEIPL	128.056	31
3	81	IMQKPRCGV	85.394	32
4	10	CLLPGSLAL	79.041	33
5	60	KEMQKFFGL	59.278	34
6	203	SLGINFLYA	51.916	35

7	73	MLNSRVIEI	40.792	36
8	4	TVLCAVCLL	15.907	37
9	132	ALNMWGKEI	10.433	38
10	109	VTYRIVSYT	7.122	39
11	127	RLVSKALNM	4.968	40
12	154	IMIGFARGA	4.636	41
13	43	YLYDSETKN	4.497	42
14	140	IPLHFRKVV	4.449	43
15	146	KVVWGTADI	3.195	44
16	36	QDYLKRFYL	3.029	45
17	2	RLTVLCAVC	2.037	46
18	201	GSSLGINFL	1.764	47
19	70	ITGMLNSRV	1.642	48
20	205	GINFLYAAT	1.537	49
HLA A0205				
1	4	TVLCAVCLL	84.000	50
2	208	FLYAATHEL	63.000	51
3	60	KEMQKFFGL	21.168	52
4	10	CLLPGSLAL	21.000	53
5	134	NMWGKEIPL	21.000	54
6	8	AVCLLPGSL	14.000	55
7	146	KVVWGTADI	6.000	56
8	73	MLNSRVIEI	3.400	57
9	81	IMQKPRCGV	3.400	58
10	66	FGLPITGML	3.150	59
11	147	VVWGTADIM	2.550	60
12	212	ATHELGHSL	2.100	61
13	178	HAFAPGTGL	2.100	62

14	205	GINFLYAAT	2.000	63
15	22	QEAGGMSEL	1.960	64
16	112	RIVSYTRDL	1.680	65
17	167	YPFDGPGNT	1.350	66
18	3	LTVLCAVCL	1.190	67
19	140	IPLHFRKVV	1.020	68
20	109	VTYRIVSYT	1.020	69
HLA A1				
1	225	SSDPNAVMY	750.000	70
2	78	VIEIMQKPR	9.000	71
3	198	WTDGSSLGI	6.250	72
4	238	NGDPQNFKL	6.250	73
5	92	VAEYSLFPN	4.500	74
6	35	AQDYLKRFY	3.750	75
7	202	SSLGINFLY	3.750	76
8	46	DSETKNANS	2.700	77
9	87	CGVPDVAEY	2.500	78
10	248	QDDIKGIQK	2.500	79
11	27	MSELQWEQA	1.350	80
12	150	GTADIMIGF	1.250	81
13	123	ITVDRLVSK	1.000	82
14	108	VVTYRIVSY	1.000	83
15	54	SLEAKLKEM	0.900	84
16	163	HGDSYPFDG	0.625	85
17	10	CLLPGSLAL	0.500	86
18	151	TADIMIGFA	0.500	87
19	138	KEIPLHFRK	0.500	88
20	137	GKEIPLHFR	0.450	89

HLA A24

1	115	SYTRDLPHI	50.000	90
2	112	RIVSYTRDL	12.000	91
3	66	FGLPITGML	10.080	92
4	37	DYLKRFYLY	9.000	93
5	51	NANSLEAKL	7.920	94
6	166	SYPFKGPGN	7.500	95
7	10	CLLPGSLAL	7.200	96
8	4	TVLCAVCLL	6.000	97
9	31	QWEQAQDYL	6.000	98
10	3	LTVLCAVCL	6.000	99
11	212	ATHELGHSL	5.760	100
12	238	NGDPQNFKL	5.280	101
13	44	LYDSETKNA	5.000	102
14	235	TYGNGDPQN	5.000	103
15	243	NFKLSQDDI	5.000	104
16	8	AVCLLPGSL	4.800	105
17	12	LPGSLALPL	4.800	106
18	58	KLKEMQKFF	4.800	107
19	201	GSSLGINFL	4.800	108
20	208	FLYAATHEL	4.400	109

HLA B7

1	120	LPHITVDRL	80.000	110
2	12	LPGSLALPL	80.000	111
3	8	AVCLLPGSL	60.000	112
4	84	KPRCGVPDV	40.000	113
5	89	VPDVAEYSL	24.000	114
6	4	TVLCAVCLL	20.000	115

7	178	HAFAPGTGL	18.000	116
8	51	NANSLEAKL	12.000	117
9	212	ATHELGHSL	12.000	118
10	140	IPLHFRKVV	6.000	119
11	147	VVWGTADIM	5.000	120
12	208	FLYAATHEL	4.000	121
13	101	SPKWTSKVV	4.000	122
14	10	CLLPGSLAL	4.000	123
15	3	LTVLCAVCL	4.000	124
16	201	GSSLGINFL	4.000	125
17	134	NMWGKEIPL	4.000	126
18	112	RIVSYTRDL	4.000	127
19	125	VDRLVSKAL	4.000	128
20	66	FGLPITGML	4.000	129
HLA B8				
1	134	NMWGKEIPL	4.000	130
2	56	EAKLKEMQK	3.200	131
3	101	SPKWTSKVV	2.400	132
4	73	MLNSRVIEI	2.000	133
5	84	KPRCGVPDV	1.200	134
6	127	RLVSKALNM	1.000	135
7	105	TSKVVTYRI	1.000	136
8	51	NANSLEAKL	0.800	137
9	12	LPGSLALPL	0.800	138
10	120	LPHITVDRL	0.800	139
11	178	HAFAPGTGL	0.800	140
12	54	SLEAKLKEM	0.400	141
13	10	CLLPGSLAL	0.400	142

14	208	FLYAATHEL	0.400	143
15	125	VDRLVSKAL	0.400	144
16	158	FARGAHGDS	0.400	145
17	36	QDYLKRFYL	0.400	146
18	212	ATHELGHSL	0.300	147
19	116	YTRDLPHIT	0.300	148
20	62	MQKFFGLPI	0.300	149
HLA B2702				
1	159	ATGAHGDSY	200.000	150
2	30	LQWEQAQDY	100.00	151
3	196	ERWTDGSSL	90.000	152
4	40	KRFYLYDSE	30.000	153
5	1	MRLTVLCAV	20.000	154
6	144	FRKVVWGTA	20.000	155
7	117	TRDLPHITV	20.000	156
8	134	NMWGKEIPL	7.500	157
9	96	SLFPNSPKW	7.500	158
10	62	MQKFFGLPI	6.000	159
11	35	AQDYLKRFY	6.000	160
12	208	FLYAATHEL	4.500	161
13	76	SRVIEIMQK	4.000	162
14	126	DRLVSKALN	3.000	163
15	60	KEMQKFFGL	2.700	164
16	58	KLKEMQKFF	2.700	165
17	256	KLYGKRSNS	2.250	166
18	85	PRCGVPDVA	2.000	167
19	111	YRIVSYTRD	2.000	168
20	178	HAFAPGTGL	1.500	169

HLA B4403

1	87	CGVPDVAEY	36.000	170
2	202	SSLGINFLY	27.000	171
3	79	IEIMQKPRC	20.000	172
4	60	KEMQKFFGL	18.000	173
5	225	SSDPNAVMY	18.000	174
6	47	SETKNANSL	12.000	175
7	195	DERWTDGSS	12.000	176
8	214	HELGHSLGM	12.000	177
9	22	QEAGGMSEL	12.000	178
10	249	DDIKGIQKL	11.250	179
11	93	AEYSLFPNS	8.000	180
12	138	KEIPLHFRK	6.000	181
13	184	TGLGGDAHF	3.000	182
14	200	DGSSLGINF	3.000	183
15	35	AQDYLKRFY	3.000	184
16	34	QAQDYLKRF	2.250	185
17	30	LQWEQAQDY	2.250	186
18	250	DIKGIQKLY	2.025	187
19	150	GTADIMIGF	2.000	188
20	37	DYLKRFYLY	1.800	189

Any patents or publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. Further, these patents and publications are incorporated by reference herein to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

One skilled in the art will appreciate readily that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those objects, ends and advantages inherent herein. The present examples, along with the methods, procedures, treatments, molecules, and specific compounds described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention as defined by the scope of the claims.

WHAT IS CLAIMED IS:

1. A method of diagnosing cancer in an individual, comprising the steps of:

(a) obtaining a biological sample from an individual; and

(b) detecting PUMP-1 in said sample, wherein the presence of PUMP-1 in said sample is indicative of the presence of cancer in said individual, wherein the absence of PUMP-1 in said sample is indicative of the absence of cancer in said individual.

2. The method of claim 1, wherein said biological sample is selected from the group consisting of blood, urine, saliva, tears, interstitial fluid, ascites fluid, tumor tissue biopsy and circulating tumor cells.

3. The method of claim 1, wherein said detection of said PUMP-1 is by means selected from the group consisting of Northern blot, Western blot, PCR, dot blot, ELIZA sandwich assay, radioimmunoassay, DNA array chips and flow cytometry.

4. The method of claim 1, wherein said cancer is selected from the group consisting of ovarian, breast, lung, colon, prostate and others in which PUMP-1 is overexpressed.

5. A method for detecting malignant hyperplasia in a biological sample, comprising the steps of:

(a) isolating mRNA from said sample; and

(b) detecting PUMP-1 mRNA in said sample, wherein

the presence of said PUMP-1 mRNA in said sample is indicative of the presence of malignant hyperplasia, wherein the absenCe of said PUMP-1 mRNA in said sample is indicative of the absence of malignant hyperplasia.

6. The method of claim 5, further comprising the step of:

comparing said PUMP-1 mRNA to reference information, wherein said comparison provides a diagnosis of said malignant hyperplasia.

7. The method of claim 5 further comprising the step of:

comparing said PUMP-1 mRNA to reference information, wherein said comparison determines a treatment of said malignant hyperplasia.

8. The method of claim 5, wherein said detection of said PUMP-1 mRNA is by PCR amplification.

9. The method of claim 8, wherein said PCR amplification uses primers selected from the group consisting of SEQ ID No. 8 and SEQ ID No. 9.

10. The method of claim 5, wherein said biological sample is selected from the group consisting of blood, urine, saliva, tears, interstitial fluid, ascites fluid, tumor tissue biopsy and circulating tumor cells.

11. A method for detecting malignant hyperplasia in a biological sample, comprising the steps of:

(a) isolating protein from said sample; and

(b) detecting PUMP-1 protein in said sample, wherein the presence of said PUMP-1 protein in said sample is indicative of the presence of malignant hyperplasia, wherein the absense of said PUMP-1 protein in said sample is indicative of the absence of malignant hyperplasia.

12. The method of claim 11, further comprising the step of:

comparing said PUMP-1 protein to reference information, wherein said comparison provides a diagnosis of said malignant hyperplasia.

13. The method of claim 11, further comprising the step of:

comparing said PUMP-1 protein to reference information, wherein said comparison determines a treatment of said malignant hyperplasia.

14. The method of claim 11, wherein said detection is by immunoaffinity to an antibody, wherein said antibody is specific for PUMP-1.

15. The method of claim 11, wherein said biological sample is selected from the group consisting of blood, urine, saliva, tears, interstitial fluid, ascites fluid, tumor tissue biopsy

and circulating tumor cells.

16. A method of inhibiting expression of endogenous PUMP-1 in a cell, comprising the step of:

introducing a vector into a cell, wherein said vector comprises a PUMP-1 gene in opposite orientation operably linked to elements necessary for expression, wherein expression of said vector in said cell produces PUMP-1 antisense mRNA, wherein said PUMP-1 antisense mRNA hybridizes to endogenous PUMP-1 mRNA, thereby inhibiting expression of endogenous PUMP-1 in said cell.

17. A method of inhibiting PUMP-1 protein in a cell, comprising the step of:

introducing an antibody into a cell, wherein said antibody is specific for a PUMP-1 protein or a fragment thereof, wherein binding of said antibody to said PUMP-1 protein inhibits said PUMP-1 protein.

18. A method of targeted therapy to an individual, comprising the step of:

administering a compound to an individual, wherein said compound has a targeting moiety and a therapeutic moiety, wherein said targeting moiety is specific for PUMP-1.

19. The method of claim 18, wherein said targeting moiety is selected from the group consisting of an antibody specific for PUMP-1 and a ligand or ligand binding domain that binds PUMP-1.

20. The method of claim 18, wherein said therapeutic moiety is selected from the group consisting of a radioisotope, a toxin, a chemotherapeutic agent, an immune stimulant and a cytotoxic agent.

21. The method of claim 18, wherein said individual suffers from a disease selected from the group consisting of ovarian cancer, lung cancer, prostate cancer, colon cancer and other cancers in which PUMP-1 is overexpressed.

22. A method of vaccinating an individual against PUMP-1, comprising the step of:

inoculating an individual with a PUMP-1 protein or fragment thereof, wherein said PUMP-1 protein or fragment thereof lack PUMP-1 protease activity, wherein said inoculation with said PUMP-1 protein or fragment thereof elicits an immune response in said individual, thereby vaccinating said individual against PUMP-1.

23. The method of claim 22, wherein said individual has cancer, is suspected of having cancer or is at risk of getting cancer.

24. The method of claim 22, wherein said PUMP-1 fragment is selected from the group consisting of a 9-residue fragment up to a 20-residue fragment.

25. The method of claim 24, wherein said 9-residue fragment is selected from the group consisting of SEQ ID Nos. 30, 31, 32, 33, 50, 70, 110, 111, 150, 151 and 152.

26. A method of producing immune-activated cells directed toward PUMP-1, comprising the steps of:

exposing dendritic cells to a PUMP-1 protein or fragment thereof, wherein said PUMP-1 protein or fragment thereof lacks PUMP-1 protease activity, wherein said exposure to said PUMP-1 protein or fragment thereof activates said dendritic cells, thereby producing immune-activated cells directed toward PUMP-1.

27. The method of claim 26, wherein said immune-activated cells are selected from the group consisting of B-cells, T-cells and dendrites.

28. The method of claim 26, wherein said PUMP-1 fragment is selected from the group consisting of a 9-residue fragment up to a 20-residue fragment.

29. The method of claim 28, wherein said 9-residue fragment is selected from the group consisting of SEQ ID Nos. 30, 31, 32, 33, 50, 70, 110, 111, 150, 151 and 152.

30. The method of claim 26, wherein said dendritic cells are isolated from an individual prior to said exposure, wherein said activated dendritic cells are reintroduced into said

individual subsequent to said exposure.

31. The method of claim 30, wherein said individual has cancer, is suspected of having cancer or is at risk of getting cancer.

32. An immunogenic composition, comprising an immunogenic fragment of a PUMP-1 protein and an appropriate adjuvant.

33. The immunogenic composition of claim 32, wherein said fragment is selected from the group consisting of a 9-residue fragment up to a 20-residue fragment.

34. The immunogenic composition of claim 33, wherein said 9-residue fragment is selected from the group consisting of SEQ ID Nos. 30, 31, 32, 33, 50, 70, 110, 111, 150, 151 and 152.

35. An oligonucleotide having a sequence complementary to SEQ ID No. 29.

36. A composition comprising the oligonucleotide of claim 35 and a physiologically acceptable carrier.

37. A method of treating a neoplastic state in an individual in need of such treatment, comprising the step of: administering to said individual an effective dose of the oligonucleotide of claim 35.

38. The method of claim 37, wherein said neoplastic state is selected from the group consisting of ovarian cancer, breast cancer, lung cancer, colon cancer, prostate cancer and other cancers in which PUMP-1 is overexpressed.

39. A method of screening for compounds that inhibit PUMP-1 activity, comprising the steps of:

contacting a sample with a compound, wherein said sample comprises PUMP-1 protein; and

assaying for PUMP-1 protease activity, wherein a decrease in said PUMP-1 protease activity in the presence of said compound relative to PUMP-1 protease activity in the absence of said compound indicatives the compound inhibits PUMP-1 activity.

40. A method for detecting ovarian malignant hyperplasia in a biological sample, comprising the steps of:

(a) isolating the proteases or protease mRNA present in said biological sample; and

(b) detecting specific proteases or protease mRNA present in said biological sample, wherein said proteases are selected from the group consisting of hepsin, protease M, complement factor B, SCCE, other serine proteases indicated in lanes 2 and 4 of Figure 1, cathepsin L and PUMP-1.

41. The method of claim 40, further comprising the step of: comparing the specific proteases or protease mRNA detected to reference information, wherein said comparison

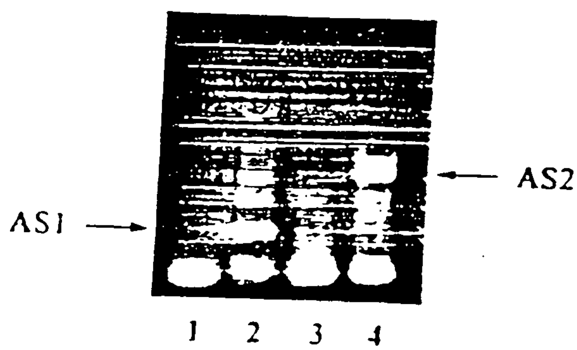
provides a diagnoses of said malignant hyperplasia.

42. The method of claim 40, further comprising the step of: comparing the specific proteases or protease mRNA detected to reference information, wherein said comparison determines a treatment of said malignant hyperplasia.

43. The method of claim 40, wherein said protease mRNA is detected by amplification of total mRNA.

44. The method of claim 40, wherein said protease is detected with an antibody.

45. The method of claim 40, wherein said biological sample is selected from the group consisting of blood, urine, saliva, tears, interstitial fluid, ascites fluid, tumor tissue biopsy and circulating tumor cells.



1) Normal Ovary 2) Tumor
3) Normal Ovary 4) Tumor

Fig. 1

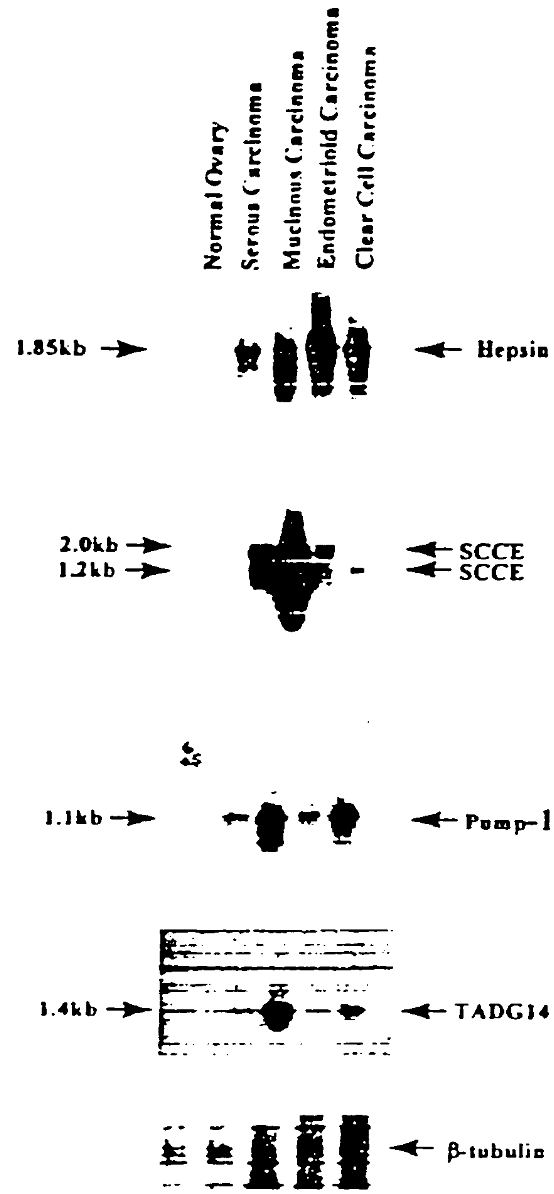

Normal Ovary
Serous Carcinoma
Mucinous Carcinoma
Endometrioid Carcinoma
Clear Cell Carcinoma
1.85kb
Hepsin
2.0kb
1.2kb
SCCE
SCCE
1.1kb
Pump-1
1.4kb
TADG14
β-tubulin

Fig. 2

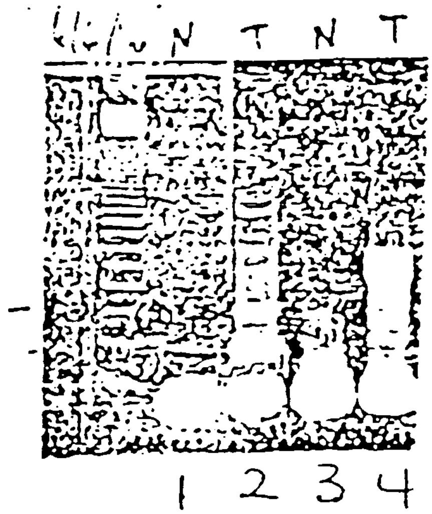


Fig. 3

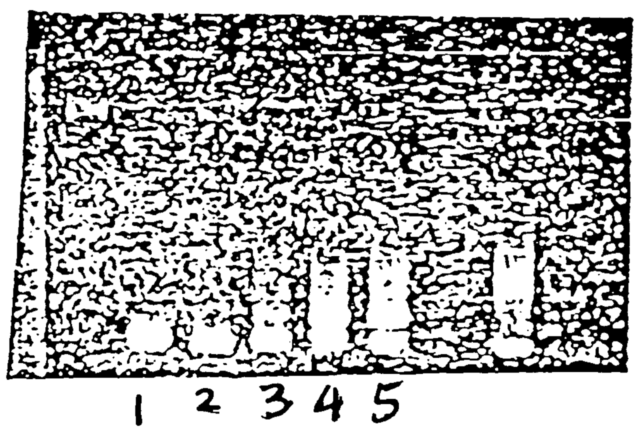


Fig. 4

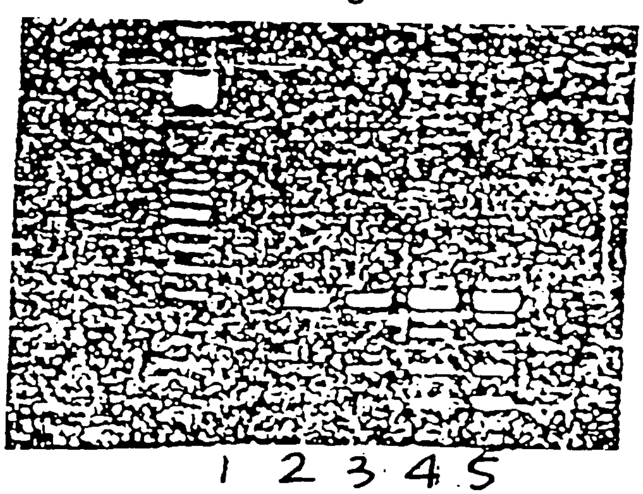


Fig. 5

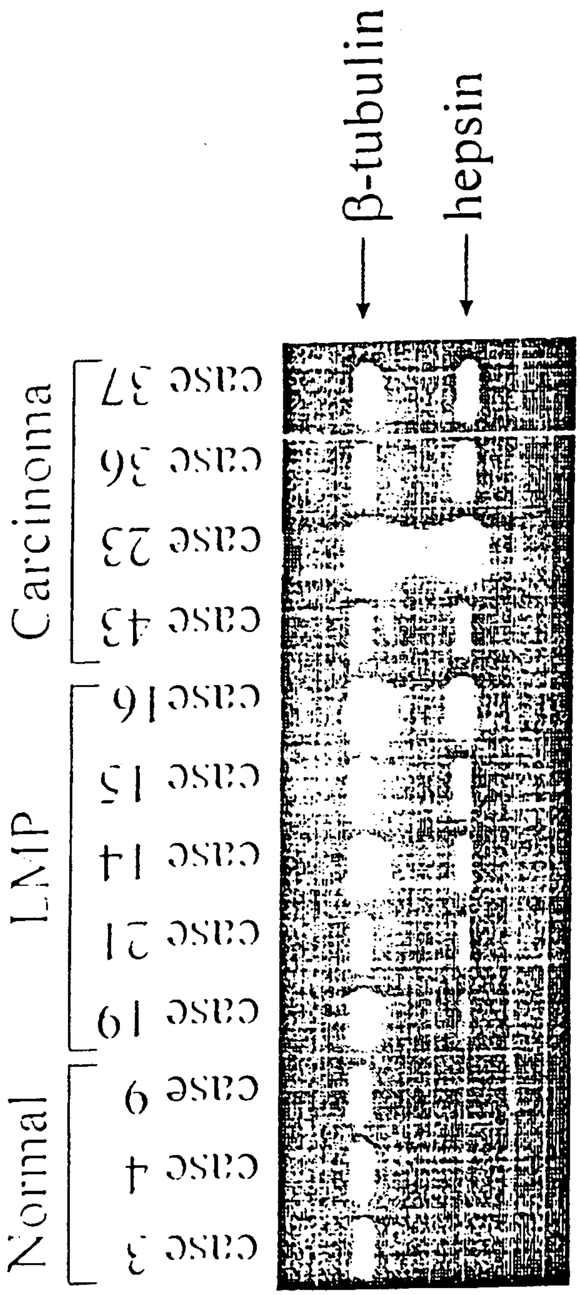

β-tubulin
hepsin
Normal
case 3
case 4
case 9
LMP
case 19
case 21
case 14
case 15
case16
Carcinoma
case 43
case 23
case 36
case 37

Fig. 6

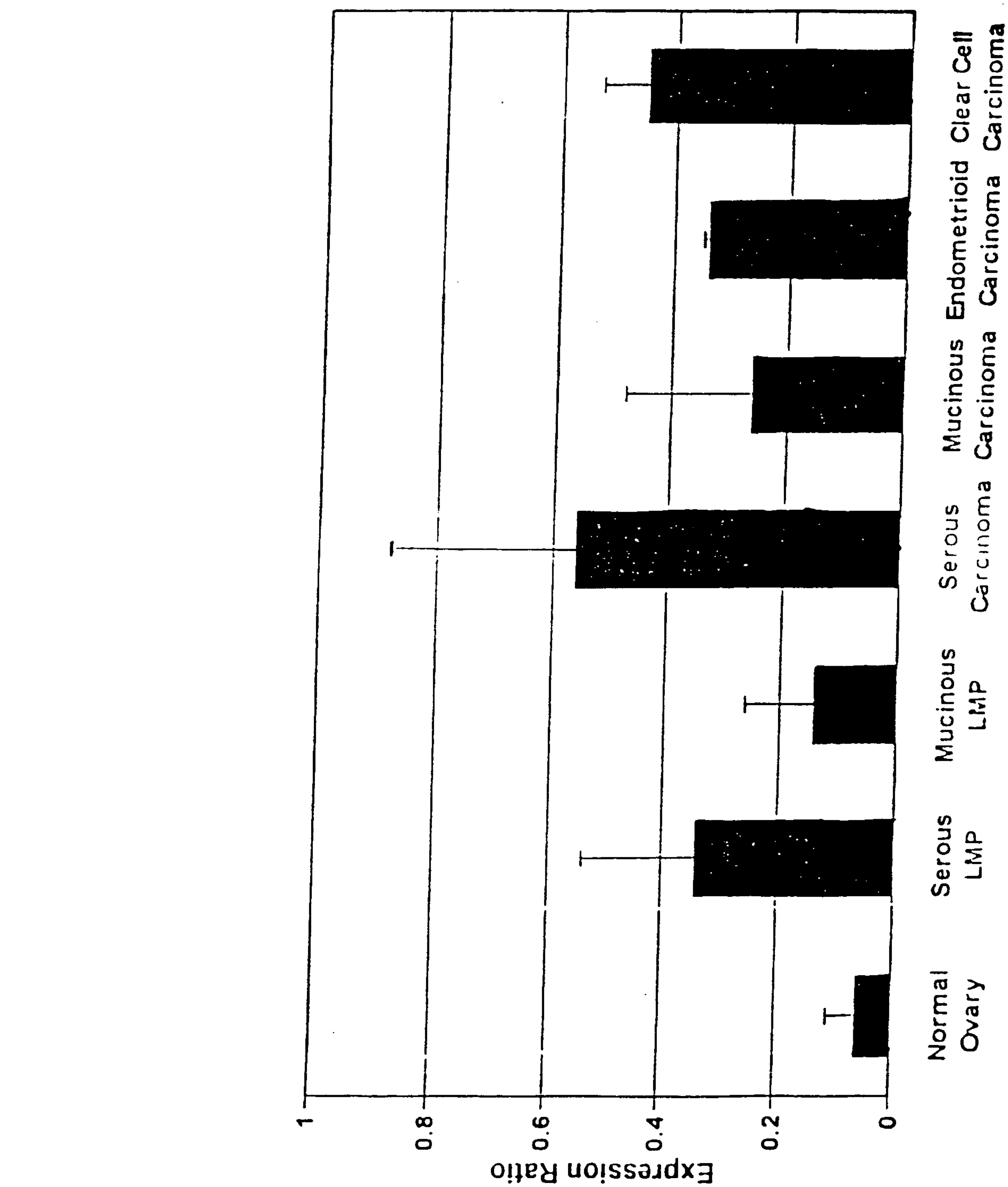

Expression Ratio
0
0.2
0.4
0.6
0.8
1
Normal Ovary
Serous LMP
Mucinous LMP
Serous Carcinoma
Mucinous Carcinoma
Endometrioid Carcinoma
Clear Cell Carcinoma

Fig. 7

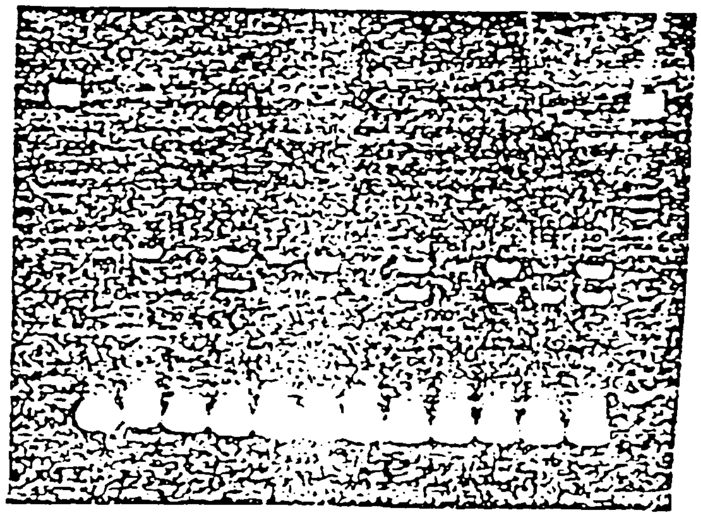
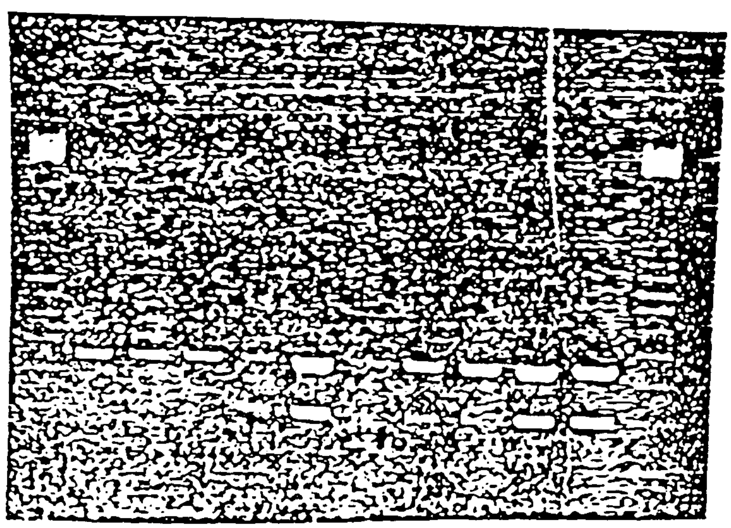


Fig. 8



1 2 3 4 5 6 7 8 9 10

Fig. 9

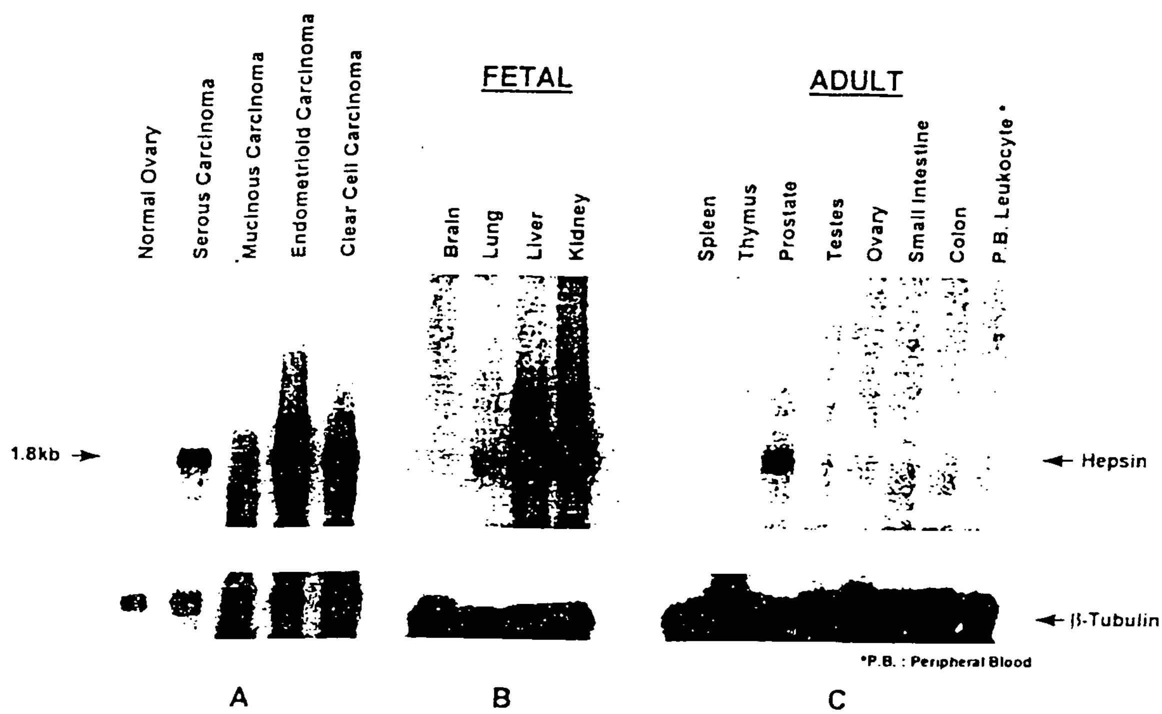

FETAL
ADULT
Normal Ovary
Serous Carcinoma
Mucinous Carcinoma
Endometrioid Carcinoma
Clear Cell Carcinoma
Brain
Lung
Liver
Kidney
Spleen
Thymus
Prostate
Testes
Ovary
Small Intestine
Colon
P.B. Leukocyte *
1.8kb
Hepsin
β-Tubulin
*P.B. : Peripheral Blood
A
B
C

Fig. 10

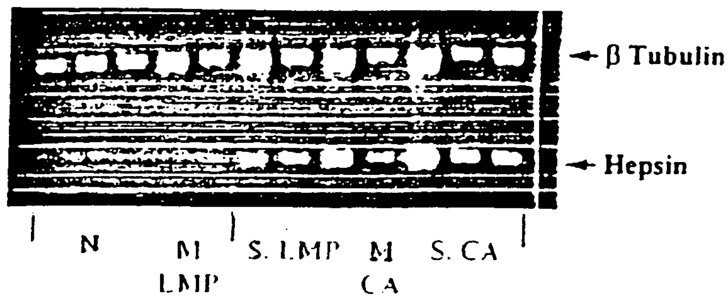


Fig. 11A

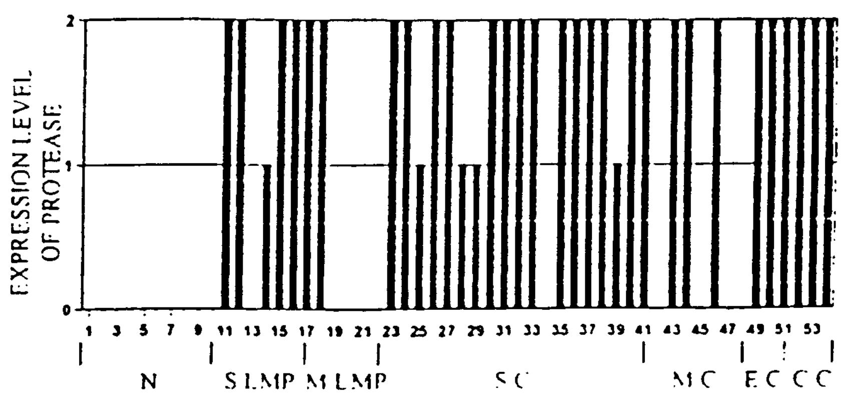


Fig. 11B

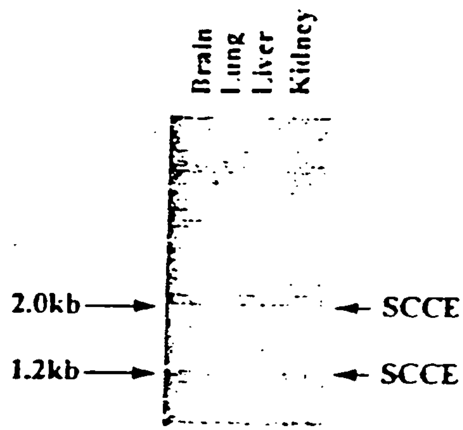


Fig. 12A

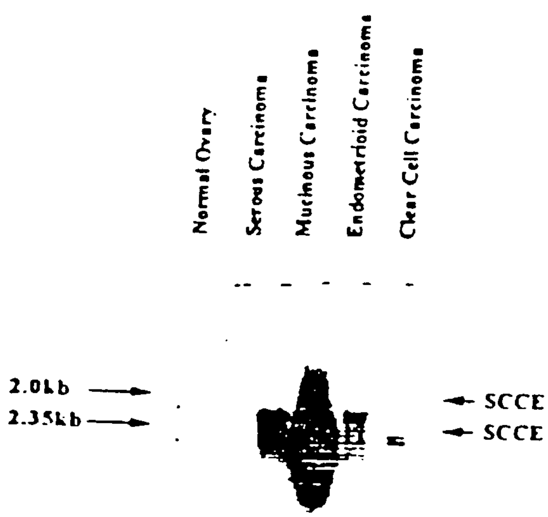


Fig. 12B

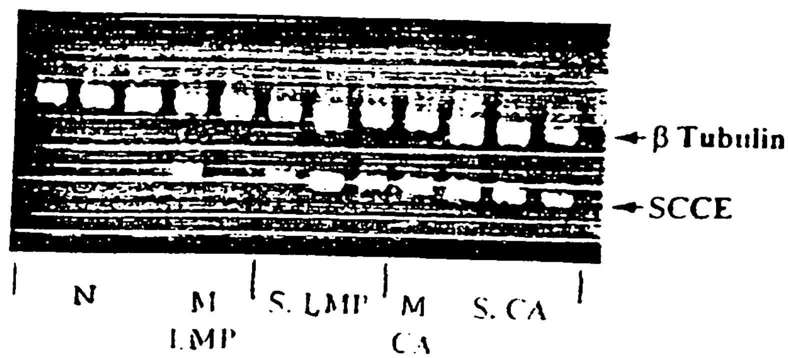


Fig. 13A

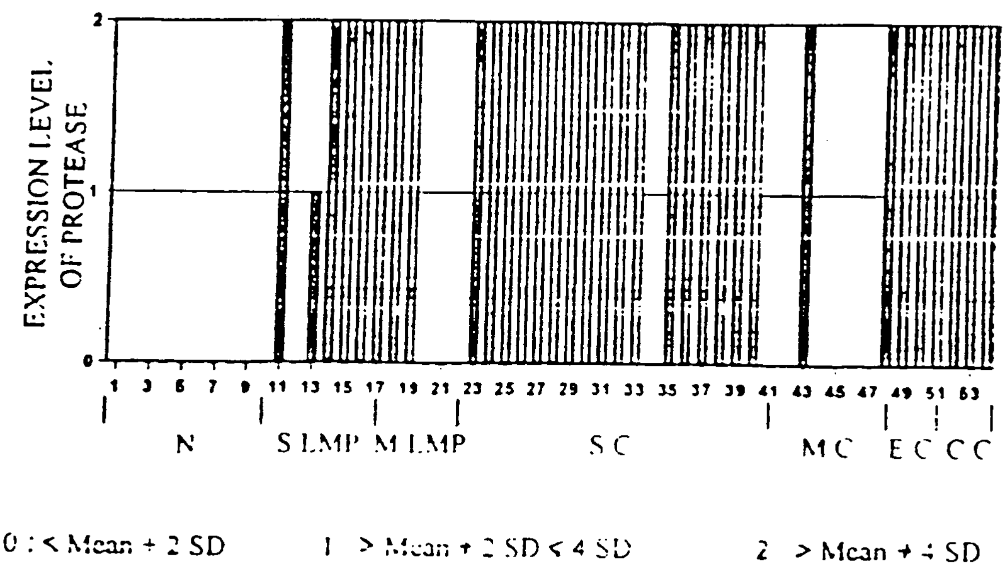


Fig. 13B

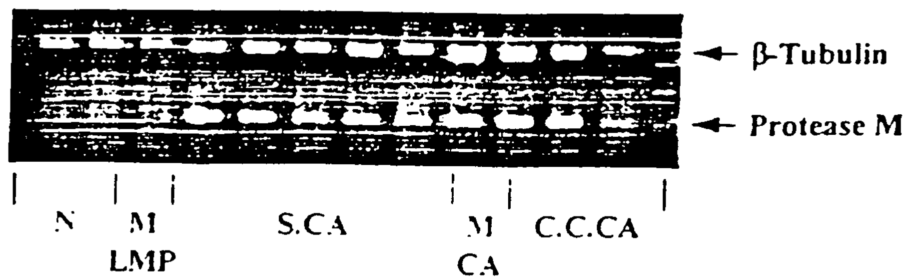


Fig. 14

1. .15

VVTAAHCVYDLYLPK

16 .30

SWTIQVGLVSLLDNP

↓ indicates the site of insert in TADG12

31 .45

H & D are the conserved regions of Serine protease.

APSHLVEKIVYHSKY

46 57

KPKRLGNDIALL

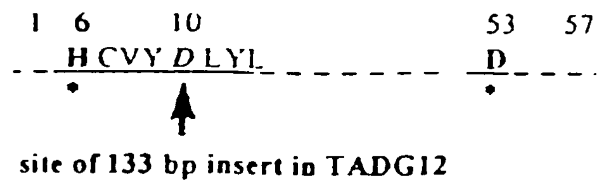


Fig. 15

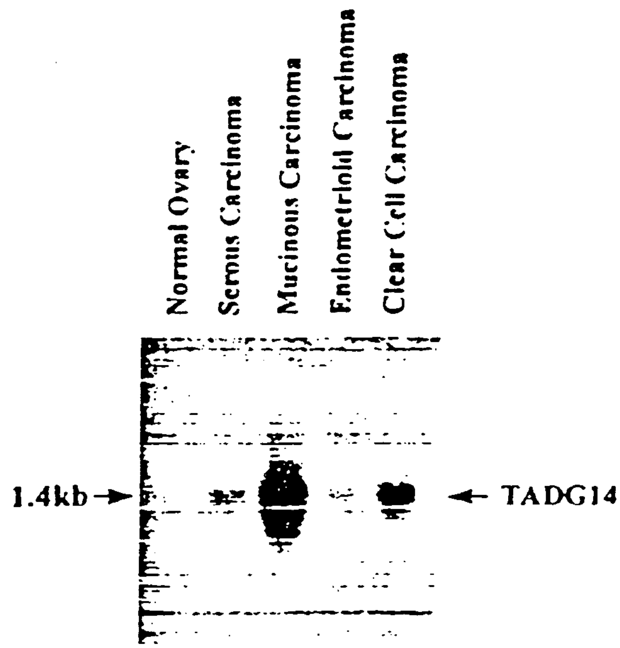


Fig. 16A

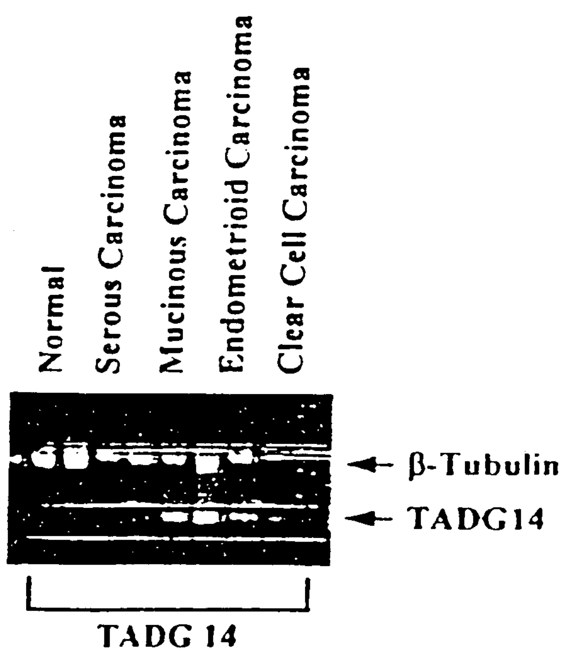
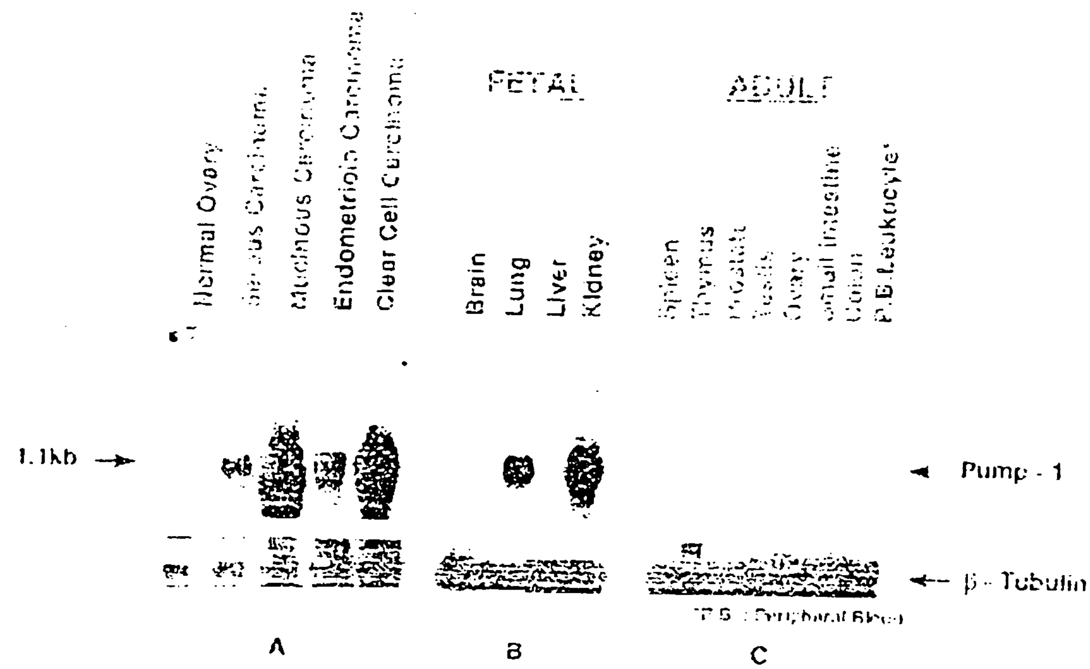


Fig. 16B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ADULT
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Thymus
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Colon
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1.1kb
Pump - 1
β - Tubulin
A
B
C

Fig. 17

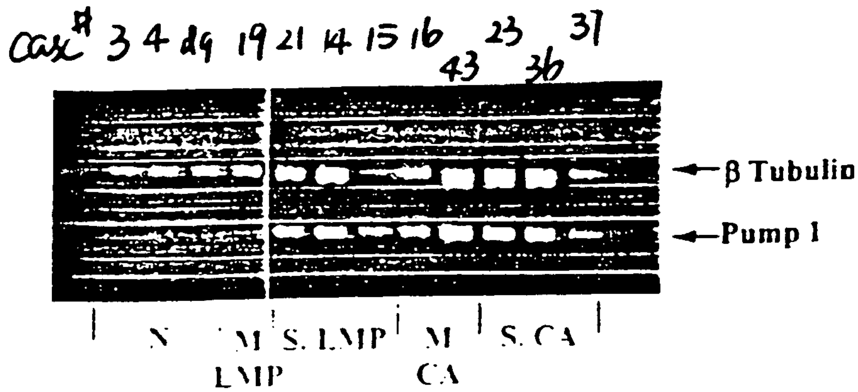


Fig. 18A

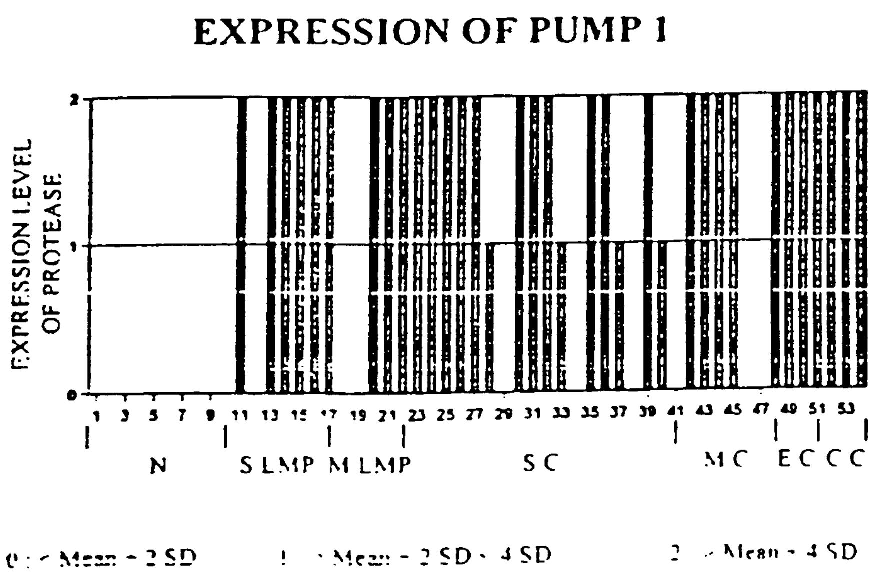


Fig. 18B

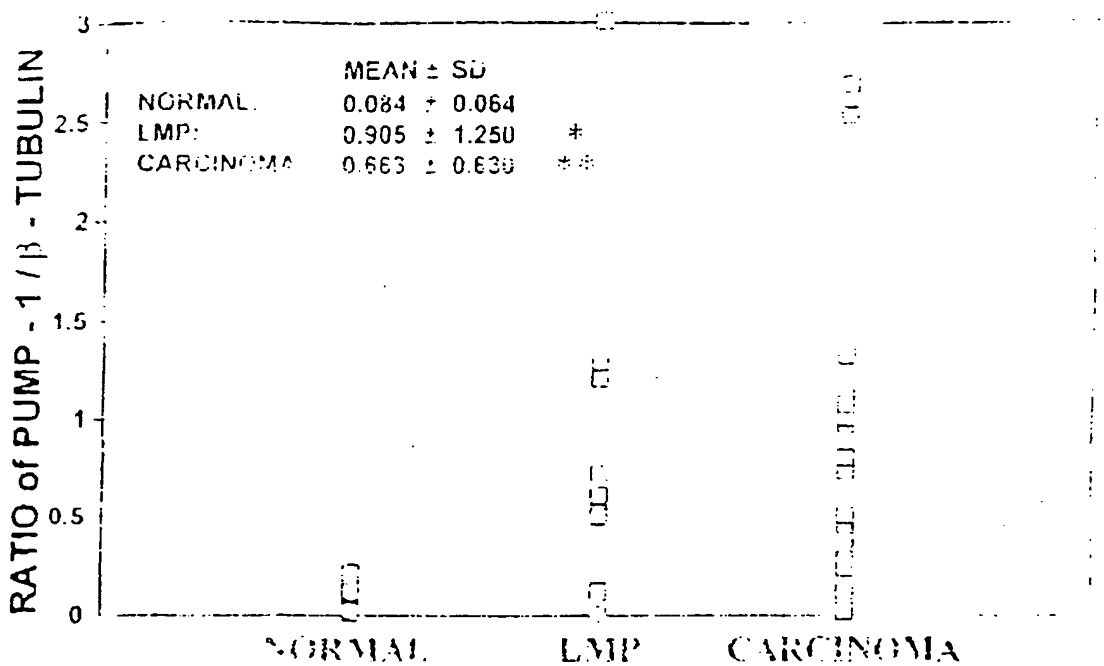

RATIO of PUMP - 1 / β - TUBULIN
MEAN ± SD
NORMAL 0.084 ± 0.064
LMP: 0.905 ± 1.250 *
CARCINOMA 0.663 ± 0.630 **
0
0.5
1
1.5
2
2.5
3
NORMAL
LMP
CARCINOMA

Fig. 19

Fig. 20A

Fig. 20B

Fig. 20C

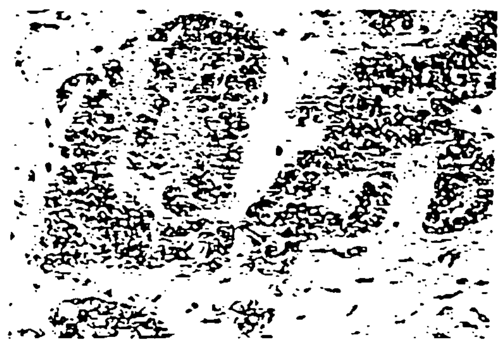
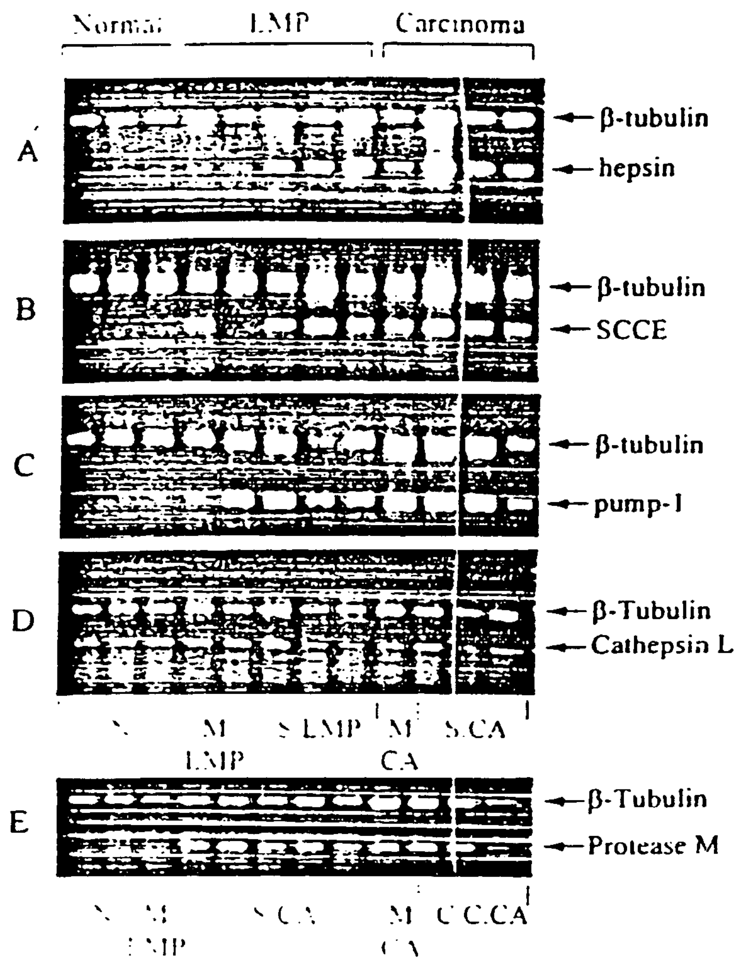


Fig. 20D

Fig. 20E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LMP
Carcinoma
A
β-tubulin
hepsin
B
β-tubulin
SCCE
C
β-tubulin
pump-1
D
β-Tubulin
Cathepsin L
S.CA
CA
E
β-Tubulin
Protease M
C.CA

Fig. 21

SEQUENCE LISTING

<110> O'Brien, Timothy J.

<120> Compositions and Methods for the Early Diagnosis of Ovarian Cancer

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<210> 14
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<400> 15
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<210> 21
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<400> 21
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<210> 23
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<220>
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<400> 23
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<210> 24
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<220>
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<400> 25
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<210> 26
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<220>
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<400> 26

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<220>
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<400> 27
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uuuaaagccu uugacacuaa ucgauccacu guaauaugcg guaagucucg 700
aguauaugau acgauccugu aggugaccac uuuggaaguc cauuuugggc 750
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uagcugccgu ccagagacaa uuguucuu 1078

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<220>
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<210> 30
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
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<210> 31
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 134-142 of the PUMP-1 protein

<400> 31

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Asn Met Trp Gly Lys Glu Ile Pro Leu
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<210> 32
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 81-89 of the PUMP-1 protein

<400> 32
Ile Met Gln Lys Pro Arg Cys Gly Val
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<210> 33
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 10-18 of the PUMP-1 protein

<400> 33
Cys Leu Leu Pro Gly Ser Leu Ala Leu
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<210> 34
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 60-68 of the PUMP-1 protein

<400> 34
Lys Glu Met Gln Lys Phe Phe Gly Leu
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<210> 35
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 203-211 of the PUMP-1 protein

<400> 35
Ser Leu Gly Ile Asn Phe Leu Tyr Ala
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<210> 36
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 73-81 of the PUMP-1 protein

<400> 36
Met Leu Asn Ser Arg Val Ile Glu Ile
5

<210> 37
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 4-12 of the PUMP-1 protein

<400> 37
Thr Val Leu Cys Ala Val Cys Leu Leu
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<210> 38
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 132-140 of the PUMP-1 protein

<400> 38
Ala Leu Asn Met Trp Gly Lys Glu Ile
5

<210> 39
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 109-117 of the PUMP-1 protein

<400> 39
Val Thr Tyr Arg Ile Val Ser Tyr Thr
5

<210> 40
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 127-135 of the PUMP-1 protein

<400> 40
Arg Leu Val Ser Lys Ala Leu Asn Met
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<210> 41
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>

<223> Residues 154-162 of the PUMP-1 protein

<400> 41
Ile Met Ile Gly Phe Ala Arg Gly Ala
5

<210> 42
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 43-51 of the PUMP-1 protein

<400> 42
Tyr Leu Tyr Asp Ser Glu Thr Lys Asn
5

<210> 43
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 140-148 of the PUMP-1 protein

<400> 43
Ile Pro Leu His Phe Arg Lys Val Val
5

<210> 44
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 146-154 of the PUMP-1 protein

<400> 44
Lys Val Val Trp Gly Thr Ala Asp Ile
5

<210> 45
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 36-44 of the PUMP-1 protein

<400> 45
Gln Asp Tyr Leu Lys Arg Phe Tyr Leu
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<210> 46
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 2-10 of the PUMP-1 protein

<400> 46
Arg Leu Thr Val Leu Cys Ala Val Cys
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<210> 47
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 201-209 of the PUMP-1 protein

<400> 47
Gly Ser Ser Leu Gly Ile Asn Phe Leu
5

<210> 48
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 70-78 of the PUMP-1 protein

<400> 48
Ile Thr Gly Met Leu Asn Ser Arg Val
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<210> 49
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
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Gly Ile Asn Phe Leu Tyr Ala Ala Thr
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<210> 50
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 4-12 of the PUMP-1 protein

<400> 50
Thr Val Leu Cys Ala Val Cys Leu Leu
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<210> 51
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 208-216 of the PUMP-1 protein

<400> 51
Phe Leu Tyr Ala Ala Thr His Glu Leu
5

<210> 52
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 60-68 of the PUMP-1 protein

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Lys Glu Met Gln Lys Phe Phe Gly Leu
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<210> 53
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 10-18 of the PUMP-1 protein

<400> 53
Cys Leu Leu Pro Gly Ser Leu Ala Leu
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<210> 54
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
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Asn Met Trp Gly Lys Glu Ile Pro Leu
5

<210> 55
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 8-16 of the PUMP-1 protein

<400> 55
Ala Val Cys Leu Leu Pro Gly Ser Leu
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<210> 56
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<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 146-154 of the PUMP-1 protein

<400> 56
Lys Val Val Trp Gly Thr Ala Asp Ile
5

<210> 57
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 73-81 of the PUMP-1 protein

<400> 57
Met Leu Asn Ser Arg Val Ile Glu Ile
5

<210> 58
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 81-89 of the PUMP-1 protein

<400> 58
Ile Met Gln Lys Pro Arg Cys Gly Val
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<210> 59
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 66-74 of the PUMP-1 protein

<400> 59
Phe Gly Leu Pro Ile Thr Gly Met Leu
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<210> 60
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 147-155 of the PUMP-1 protein

<400> 60
Val Val Trp Gly Thr Ala Asp Ile Met
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<210> 61
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 212-220 of the PUMP-1 protein

<400> 61
Ala Thr His Glu Leu Gly His Ser Leu
5

<210> 62
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 178-186 of the PUMP-1 protein

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His Ala Phe Ala Pro Gly Thr Gly Leu
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<210> 63
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 205-213 of the PUMP-1 protein

<400> 63
Gly Ile Asn Phe Leu Tyr Ala Ala Thr
5

<210> 64
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 22-30 of the PUMP-1 protein

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Gln Glu Ala Gly Gly Met Ser Glu Leu
5

<210> 65
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 112-120 of the PUMP-1 protein

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Arg Ile Val Ser Tyr Thr Arg Asp Leu
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<210> 66
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 167-175 of the PUMP-1 protein

<400> 66
Tyr Pro Phe Asp Gly Pro Gly Asn Thr
5

<210> 67
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 3-11 of the PUMP-1 protein

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Leu Thr Val Leu Cys Ala Val Cys Leu
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<210> 68
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 140-148 of the PUMP-1 protein

<400> 68
Ile Pro Leu His Phe Arg Lys Val Val
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<210> 69
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 109-117 of the PUMP-1 protein

<400> 69
Val Thr Tyr Arg Ile Val Ser Tyr Thr
5

<210> 70
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 225-233 of the PUMP-1 protein

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Ser Ser Asp Pro Asn Ala Val Met Tyr
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<210> 71
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 78-86 of the PUMP-1 protein

<400> 71
Val Ile Glu Ile Met Gln Lys Pro Arg
5

<210> 72
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 198-206 of the PUMP-1 protein

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Trp Thr Asp Gly Ser Ser Leu Gly Ile
5

<210> 73
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 238-246 of the PUMP-1 protein

<400> 73
Asn Gly Asp Pro Gln Asn Phe Lys Leu
5

<210> 74
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 92-100 of the PUMP-1 protein

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Val Ala Glu Tyr Ser Leu Phe Pro Asn
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<210> 75
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 35-43 of the PUMP-1 protein

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<210> 76
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 202-210 of the PUMP-1 protein

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Ser Ser Leu Gly Ile Asn Phe Leu Tyr
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<210> 77
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 46-54 of the PUMP-1 protein

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Asp Ser Glu Thr Lys Asn Ala Asn Ser
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<210> 78
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 87-95 of the PUMP-1 protein

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Cys Gly Val Pro Asp Val Ala Glu Tyr
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<210> 79
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 248-256 of the PUMP-1 protein

<400> 79
Gln Asp Asp Ile Lys Gly Ile Gln Lys
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<210> 80
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 27-35 of the PUMP-1 protein

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<210> 81
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 150-158 of the PUMP-1 protein

<400> 81
Gly Thr Ala Asp Ile Met Ile Gly Phe
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<210> 82
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 123-131 of the PUMP-1 protein

<400> 82
Ile Thr Val Asp Arg Leu Val Ser Lys
5

<210> 83
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 108-116 of the PUMP-1 protein

<400> 83
Val Val Thr Tyr Arg Ile Val Ser Tyr
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<210> 84
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 54-62 of the PUMP-1 protein

<400> 84
Ser Leu Glu Ala Lys Leu Lys Glu Met
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<210> 85
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 163-171 of the PUMP-1 protein

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His Gly Asp Ser Tyr Pro Phe Asp Gly
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<210> 86
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 10-18 of the PUMP-1 protein

<400> 86
Cys Leu Leu Pro Gly Ser Leu Ala Leu
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<210> 87
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 151-159 of the PUMP-1 protein

<400> 87
Thr Ala Asp Ile Met Ile Gly Phe Ala
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<210> 88
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 138-146 of the PUMP-1 protein

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Lys Glu Ile Pro Leu His Phe Arg Lys
5

<210> 89
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 137-145 of the PUMP-1 protein

<400> 89
Gly Lys Glu Ile Pro Leu His Phe Arg
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<210> 90
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 115-123 of the PUMP-1 protein

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Ser Tyr Thr Arg Asp Leu Pro His Ile
5

<210> 91
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 112-120 of the PUMP-1 protein

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Arg Ile Val Ser Tyr Thr Arg Asp Leu
5

<210> 92
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 66-74 of the PUMP-1 protein

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Phe Gly Leu Pro Ile Thr Gly Met Leu
5

<210> 93
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 37-45 of the PUMP-1 protein

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Asp Tyr Leu Lys Arg Phe Tyr Leu Tyr
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<210> 94
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 51-59 of the PUMP-1 protein

<400> 94
Asn Ala Asn Ser Leu Glu Ala Lys Leu
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<210> 95
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 166-174 of the PUMP-1 protein

<400> 95
Ser Tyr Pro Phe Lys Gly Pro Gly Asn
5

<210> 96
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 10-18 of the PUMP-1 protein

<400> 96
Cys Leu Leu Pro Gly Ser Leu Ala Leu
5

<210> 97
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 4-12 of the PUMP-1 protein

<400> 97
Thr Val Leu Cys Ala Val Cys Leu Leu
5

<210> 98
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 31-39 of the PUMP-1 protein

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Gln Trp Glu Gln Ala Gln Asp Tyr Leu
5

<210> 99
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 3-11 of the PUMP-1 protein

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Leu Thr Val Leu Cys Ala Val Cys Leu
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<210> 100
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 212-220 of the PUMP-1 protein

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Ala Thr His Glu Leu Gly His Ser Leu
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<210> 101
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 238-246 of the PUMP-1 protein

<400> 101
Asn Gly Asp Pro Gln Asn Phe Lys Leu
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<210> 102
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<213> *Homo sapiens*

<220>
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<210> 103
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
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Thr Tyr Gly Asn Gly Asp Pro Gln Asn
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<210> 104
<211> 9

<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 243-251 of the PUMP-1 protein

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Asn Phe Lys Leu Ser Gln Asp Asp Ile
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<210> 105
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 8-16 of the PUMP-1 protein

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Ala Val Cys Leu Leu Pro Gly Ser Leu
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<210> 106
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
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Leu Pro Gly Ser Leu Ala Leu Pro Leu
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<210> 107
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 58-66 of the PUMP-1 protein

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Lys Leu Lys Glu Met Gln Lys Phe Phe
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<210> 108
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 201-209 of the PUMP-1 protein

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Gly Ser Ser Leu Gly Ile Asn Phe Leu
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<210> 109
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 208-216 of the PUMP-1 protein

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Phe Leu Tyr Ala Ala Thr His Glu Leu
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<210> 110
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 120-128 of the PUMP-1 protein

<400> 110
Leu Pro His Ile Thr Val Asp Arg Leu
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<210> 111
<211> 9
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<213> *Homo sapiens*

<220>
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Leu Pro Gly Ser Leu Ala Leu Pro Leu
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<210> 112
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
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<210> 113
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
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<210> 114
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
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Val Pro Asp Val Ala Glu Tyr Ser Leu
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<210> 115
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
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<210> 116
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
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<210> 117
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>

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<210> 118
<211> 9
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<213> *Homo sapiens*

<220>
<223> Residues 212-220 of the PUMP-1 protein

<400> 118
Ala Thr His Glu Leu Gly His Ser Leu
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<210> 119
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 140-148 of the PUMP-1 protein

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Ile Pro Leu His Phe Arg Lys Val Val
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<210> 120
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<212> PRT
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<220>
<223> Residues 147-155 of the PUMP-1 protein

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<210> 121
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<212> PRT
<213> *Homo sapiens*

<220>
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<400> 121
Phe Leu Tyr Ala Ala Thr His Glu Leu
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<210> 122
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
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<400> 122
Ser Pro Lys Trp Thr Ser Lys Val Val
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<210> 123
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
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Cys Leu Leu Pro Gly Ser Leu Ala Leu
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<210> 124
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 3-11 of the PUMP-1 protein

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Leu Thr Val Leu Cys Ala Val Cys Leu
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<210> 125
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 201-209 of the PUMP-1 protein

<400> 125
Gly Ser Ser Leu Gly Ile Asn Phe Leu
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<210> 126
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 134-142 of the PUMP-1 protein

<400> 126
Asn Met Trp Gly Lys Glu Ile Pro Leu
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<210> 127
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 112-120 of the PUMP-1 protein

<400> 127
Arg Ile Val Ser Tyr Thr Arg Asp Leu
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<210> 128
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 125-133 of the PUMP-1 protein

<400> 128
Val Asp Arg Leu Val Ser Lys Ala Leu
5

<210> 129
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 66-74 of the PUMP-1 protein

<400> 129
Phe Gly Leu Pro Ile Thr Gly Met Leu
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<210> 130
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 134-142 of the PUMP-1 protein

<400> 130
Asn Met Trp Gly Lys Glu Ile Pro Leu
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<210> 131
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 56-64 of the PUMP-1 protein

<400> 131
Glu Ala Lys Leu Lys Glu Met Gln Lys
5

<210> 132
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 101-109 of the PUMP-1 protein

<400> 132
Ser Pro Lys Trp Thr Ser Lys Val Val
5

<210> 133
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 73-81 of the PUMP-1 protein

<400> 133
Met Leu Asn Ser Arg Val Ile Glu Ile
5

<210> 134
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 84-92 of the PUMP-1 protein

<400> 134
Lys Pro Arg Cys Gly Val Pro Asp Val
5

<210> 135
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
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<400> 135
Arg Leu Val Ser Lys Ala Leu Asn Met
5

<210> 136
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 105-113 of the PUMP-1 protein

<400> 136
Thr Ser Lys Val Val Thr Tyr Arg Ile
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<210> 137
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 51-59 of the PUMP-1 protein

<400> 137
Asn Ala Asn Ser Leu Glu Ala Lys Leu
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<210> 138
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
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<400> 138
Leu Pro Gly Ser Leu Ala Leu Pro Leu
5

<210> 139
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
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<400> 139
Leu Pro His Ile Thr Val Asp Arg Leu
5

<210> 140
<211> 9
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<213> *Homo sapiens*

<220>
<223> Residues 178-186 of the PUMP-1 protein

<400> 140
His Ala Phe Ala Pro Gly Thr Gly Leu
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<210> 141
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 54-62 of the PUMP-1 protein

<400> 141
Ser Leu Glu Ala Lys Leu Lys Glu Met
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<210> 142
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
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Cys Leu Leu Pro Gly Ser Leu Ala Leu
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<210> 143
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 208-216 of the PUMP-1 protein

<400> 143
Phe Leu Tyr Ala Ala Thr His Glu Leu
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<210> 144
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 125-133 of the PUMP-1 protein

<400> 144
Val Asp Arg Leu Val Ser Lys Ala Leu
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<210> 145
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 158-166 of the PUMP-1 protein

<400> 145
Phe Ala Arg Gly Ala His Gly Asp Ser
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<210> 146
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 36-44 of the PUMP-1 protein

<400> 146
Gln Asp Tyr Leu Lys Arg Phe Tyr Leu
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<210> 147
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 212-220 of the PUMP-1 protein

<400> 147
Ala Thr His Glu Leu Gly His Ser Leu
5

<210> 148
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 116-124 of the PUMP-1 protein

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Tyr Thr Arg Asp Leu Pro His Ile Thr
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<210> 149
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 62-70 of the PUMP-1 protein

<400> 149
Met Gln Lys Phe Phe Gly Leu Pro Ile
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<210> 150
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 159-167 of the PUMP-1 protein

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Ala Thr Gly Ala His Gly Asp Ser Tyr
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<210> 151
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 30-38 of the PUMP-1 protein

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Leu Gln Trp Glu Gln Ala Gln Asp Tyr
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<210> 152
<211> 9
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<220>
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<400> 152
Glu Arg Trp Thr Asp Gly Ser Ser Leu
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<210> 153
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 40-48 of the PUMP-1 protein

<400> 153
Lys Arg Phe Tyr Leu Tyr Asp Ser Glu
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<210> 154
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 1-9 of the PUMP-1 protein

<400> 154
Met Arg Leu Thr Val Leu Cys Ala Val
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<210> 155
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
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Phe Arg Lys Val Val Trp Gly Thr Ala
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<210> 156
<211> 9
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<213> *Homo sapiens*

<220>
<223> Residues 117-125 of the PUMP-1 protein

<400> 156
Thr Arg Asp Leu Pro His Ile Thr Val
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<210> 157
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<220>
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<400> 157

Asn Met Trp Gly Lys Glu Ile Pro Leu
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<210> 158
<211> 9
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<213> *Homo sapiens*

<220>
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Ser Leu Phe Pro Asn Ser Pro Lys Trp
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<210> 159
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<220>
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Met Gln Lys Phe Phe Gly Leu Pro Ile
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<210> 160
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<220>
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<210> 161
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
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Phe Leu Tyr Ala Ala Thr His Glu Leu
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<210> 162
<211> 9

<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 76-84 of the PUMP-1 protein

<400> 162
Ser Arg Val Ile Glu Ile Met Gln Lys
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<210> 163
<211> 9
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<213> *Homo sapiens*

<220>
<223> Residues 126-134 of the PUMP-1 protein

<400> 163
Asp Arg Leu Val Ser Lys Ala Leu Asn
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<210> 164
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 60-68 of the PUMP-1 protein

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Lys Glu Met Gln Lys Phe Phe Gly Leu
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<210> 165
<211> 9
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<213> *Homo sapiens*

<220>
<223> Residues 58-66 of the PUMP-1 protein

<400> 165
Lys Leu Lys Glu Met Gln Lys Phe Phe
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<210> 166
<211> 9
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<213> *Homo sapiens*

<220>
<223> Residues 256-264 of the PUMP-1 protein

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Lys Leu Tyr Gly Lys Arg Ser Asn Ser
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<210> 167
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<220>
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Pro Arg Cys Gly Val Pro Asp Val Ala
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<210> 168
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 111-119 of the PUMP-1 protein

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Tyr Arg Ile Val Ser Tyr Thr Arg Asp
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<210> 169
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 178-186 of the PUMP-1 protein

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<210> 170
<211> 9
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<220>
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<400> 170
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<210> 171
<211> 9
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<400> 171
Ser Ser Leu Gly Ile Asn Phe Leu Tyr
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<210> 172
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<223> Residues 79-87 of the PUMP-1 protein

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<210> 173
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<213> *Homo sapiens*

<220>
<223> Residues 60-68 of the PUMP-1 protein

<400> 173
Lys Glu Met Gln Lys Phe Phe Gly Leu
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<210> 174
<211> 9
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<213> *Homo sapiens*

<220>
<223> Residues 225-233 of the PUMP-1 protein

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Ser Ser Asp Pro Asn Ala Val Met Tyr
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<210> 175
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>

<223> Residues 47-55 of the PUMP-1 protein

<400> 175
Ser Glu Thr Lys Asn Ala Asn Ser Leu
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<210> 176
<211> 9
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<213> *Homo sapiens*

<220>
<223> Residues 195-203 of the PUMP-1 protein

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Asp Glu Arg Trp Thr Asp Gly Ser Ser
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<210> 177
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 214-222 of the PUMP-1 protein

<400> 177
His Glu Leu Gly His Ser Leu Gly Met
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<210> 178
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 22-30 of the PUMP-1 protein

<400> 178
Gln Glu Ala Gly Gly Met Ser Glu Leu
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<210> 179
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 249-257 of the PUMP-1 protein

<400> 179
Asp Asp Ile Lys Gly Ile Gln Lys Leu
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<210> 180
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 93-101 of the PUMP-1 protein

<400> 180
Ala Glu Tyr Ser Leu Phe Pro Asn Ser
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<210> 181
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 138-146 of the PUMP-1 protein

<400> 181
Lys Glu Ile Pro Leu His Phe Arg Lys

<210> 182
<211> 9
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<220>
<223> Residues 184-192 of the PUMP-1 protein

<400> 182
Thr Gly Leu Gly Gly Asp Ala His Phe
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<210> 183
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 200-208 of the PUMP-1 protein

<400> 183
Asp Gly Ser Ser Leu Gly Ile Asn Phe
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<210> 184
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>

<223> Residues 35-43 of the PUMP-1 protein

<400> 184
Ala Gln Asp Tyr Leu Lys Arg Phe Tyr
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<210> 185
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<223> Residues 34-42 of the PUMP-1 protein

<400> 185
Gln Ala Gln Asp Tyr Leu Lys Arg Phe
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<210> 186
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 30-38 of the PUMP-1 protein

<400> 186
Leu Gln Trp Glu Gln Ala Gln Asp Tyr
5

<210> 187
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 250-258 of the PUMP-1 protein

<400> 187
Asp Ile Lys Gly Ile Gln Lys Leu Tyr
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<210> 188
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 150-158 of the PUMP-1 protein

<400> 188
Gly Thr Ala Asp Ile Met Ile Gly Phe

<210> 189
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 37-45 of the PUMP-1 protein

<400> 189
Asp Tyr Leu Lys Arg Phe Tyr Leu Tyr

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/02698

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.1, 7.23, 23; 424/138.1, 277.1; 530/350; 536/23.2, 23.5, 24.31, 24.33

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TANIMOTO et al. The serine proteases hepsin and stratum corneum chymotryptic enzyme (SCCE) and the metalloprotease pump-1 are overexpressed in ovarian cancer. Proceedings of the National Association for Cancer Research. March 1997, Vol. 38, page 413, abstract #2765, see entire abstract.	1-15, 40-45
X --- Y	US 5,726,015 A (MATRISIAN) 10 March 1998, columns 2-3 and 6.	1-15, 35, 36 ---------- 16-34, 37-39
Y	US 5,484,726 A (BASSET et al) 16 January 1996, col. 1 and 16.	16-34, 37-39

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
03 APRIL 2001	03 MAY 2001

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	~~CARLA MYERS~~ Joyce Bridges
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)*

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANISOWICZ et al. A novel protease homolog differentially expressed in breast and ovarian cancer. Molecular Medicine. September 1996, Vol. 2, No. 5, pages 624-636, especially page 624.	40-45
X	TANIMOTO et al. Hepsin, a cell surface serine protease is overexpressed in ovarian tumors. Society for Gynecological Investigation. 1997, Vol. 4, No. 1, page 577, see entire abstract.	40-45
X	TANIMOTO et al. Hepsin, a cell surface serine protease identified in hepatoma cells, is overexpressed in ovarian cancer. Cancer Research. July 1997, Vol. 57, No. 14, pages 2884-2887, especially pages 2884-2886.	40-45

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/02698

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

A61K 38/17, 48/00, C07H 21/04; C07K 14/435, 16/68; C12Q 1/68; G01N 33/53

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/6, 7.1, 7.23, 23; 424/138.1, 277.1; 530/350; 536/23.2, 23.5, 24.31, 24.33

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

WEST: US, EP, JP, WO Patents; DIALOG: Medline, CA, Biosis, Embase, SciSearch; EMBL, GenBank, n-gene-seq search terms: pump-1, metalloprotease, mmp-7, matrilysin, metalloproteinase, tumor or cancer, or malignant or hyperplasia, SEQ ID NO: 8, 9, 29-33, 50, 70, 110, 111, 150-152